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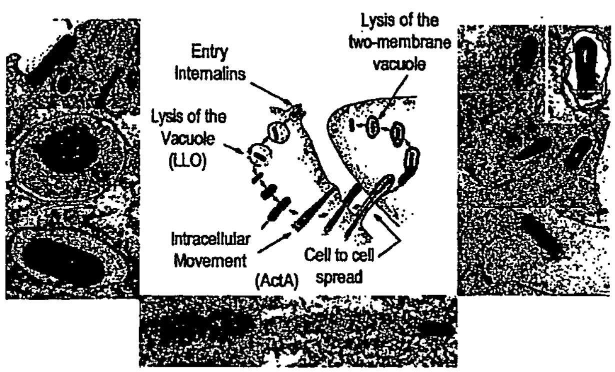

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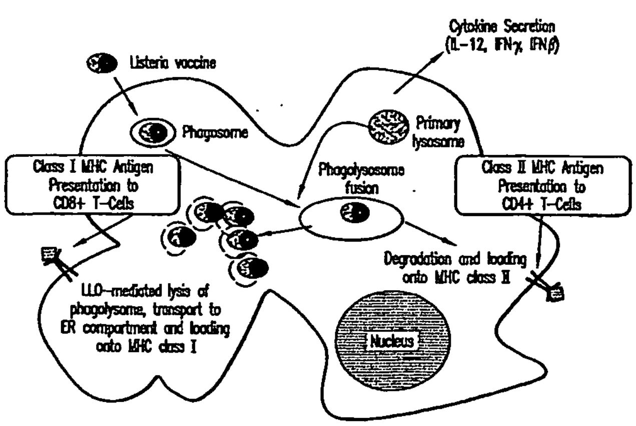

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(57) Abstract: The present invention relates to methods and compositions designed for the treatment, management, or prevention of cancer, particularly metastatic cancer and cancers of T cell origin, and hyperproliferative diseases involving EphA2-expressing cells. The methods of the invention entail the use of a *Listeria*-based EphA2 vaccine. The invention also provides pharmaceutical compositions comprising one or more *Listeria*-based vaccines of the invention either alone in combination with one or more other agents useful for cancer therapy. In certain aspects of the invention, the method entail eliciting both CD4$^+$ and CD8$^+$ T-cell responses against EphA2 and/or EphA2-expressing cells.

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WO 2005/037233 A2

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LISTERIA-BASED EphA2 VACCINES

This application claims priority to U.S. provisional application Serial No. 60/511,919, filed October 15, 2003, U.S. provisional application Serial No. 60/511,719, filed October 15, 2003, U.S. provisional application Serial No. 60/532,666, filed December 24, 2003, U.S. provisional application Serial No. 60/556,631, filed March 26, 2004, U.S. provisional application Serial No. _________, filed October 1, 2004 (Attorney Docket No. 10271-144-888), and U.S. provisional application Serial No. _________, filed October 7, 2004 (Attorney Docket No. 10271-146-888), each of which is incorporated by reference in its entirety.

1. FIELD OF THE INVENTION

[001] The present invention relates to methods and compositions for the treatment, management, or prevention of proliferative cell disease. The present invention further relates to *Listeria*-based compositions for eliciting an immune response against hyperproliferative cells and methods of using the compositions. The invention encompasses, *inter alia*, vaccines comprising *Listeria* that express an EphA2 antigenic peptide and the administration of such an EphA2 vaccine for eliciting an immune response against hyperproliferative cells that express EphA2. The invention also provides vaccines comprising one or more *Listeria*-based compositions of the invention in combination with one or more other agents useful for therapy of proliferative disorders.

2. BACKGROUND OF THE INVENTION

2.1. *Listeria*

[002] *Listeria monocytogenes* (*Listeria*) is a Gram-positive facultative intracellular bacterium that is being developed for use in antigen-specific vaccines due to its ability to prime a potent CD4+/CD8+ T-cell mediated response via both MHC class I and class II antigen presentation pathways, and as such it has been tested recently as a vaccine vector in a human clinical trial among normal healthy volunteers.

[003] *Listeria* has been studied for many years as a model for stimulating both innate and adaptive T cell-dependent antibacterial immunity. The ability of *Listeria* to effectively stimulate cellular immunity is based on its intracellular lifecycle. Upon infecting the host, the bacterium is rapidly taken up by phagocytes including macrophages and dendritic cells into a phagolysosomal compartment. The majority of the bacteria are

subsequently degraded. Peptides resulting from proteolytic degradation of pathogens within phagosomes of infected APCs are loaded directly onto MHC class II molecules, and these MHC II-peptide complexes activate CD4+ "helper" T cells that stimulate the production of antibodies, and the processed antigens are expressed on the surface of the antigen presenting cell via the class II endosomal pathway. Within the acidic compartment, certain bacterial genes are activated including the cholesterol-dependent cytolysin, LLO, which can degrade the phagolysosome, releasing the bacterium into the cytosolic compartment of the host cell, where the surviving *Listeria* propagate. Efficient presentation of heterologous antigens via the MHC class I pathway requires de novo endogenous protein expression by *Listeria*. Within antigen presenting cells (APC), proteins synthesized and secreted by *Listeria* are sampled and degraded by the proteosome. The resulting peptides are shuttled into the endoplasmic reticulum by TAP proteins and loaded onto MHC class I molecules. The MHC I-peptide complex is delivered to the cell surface, which in combination with sufficient co-stimulation (signal 2) activates and stimulates cytotoxic T lymphocytes (CTLs) having the cognate T cell receptor to expand and subsequently recognize the MHC I-peptide complex.

2.2. <u>Hyperproliferative Diseases</u>

2.2.1. <u>Cancer</u>

[004] A neoplasm, or tumor, is a neoplastic mass resulting from abnormal uncontrolled cell growth which can be benign or malignant. Benign tumors generally remain localized. Malignant tumors are collectively termed cancers. The term "malignant" generally means that the tumor can invade and destroy neighboring body structures and spread to distant sites to cause death (for review, *see* Robbins and Angell, 1976, Basic Pathology, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-122). Cancer can arise in many sites of the body and behaves differently depending upon its origin. Cancerous cells destroy the part of the body in which they originate and then spread to other part(s) of the body where they start new growth and cause more destruction.

[005] More than 1.2 million Americans develop cancer each year. Cancer is the second leading cause of death in the United States and, if current trends continue, cancer is expected to be the leading cause of death by the year 2010. Lung and prostate cancer are the top cancer killers for men in the United States. Lung and breast cancer are the top cancer killers for women in the United States. One in two men in the United States will be diagnosed with cancer at some time during his lifetime. One in three women in the United States will be diagnosed with cancer at some time during her lifetime.

[006] A cure for cancer has yet to be found. Current treatment options, such as surgery, chemotherapy and radiation treatment, are often either ineffective or present serious side effects.

2.2.2. Metastasis

[007] The most life-threatening forms of cancer often arise when a population of tumor cells gains the ability to colonize distant and foreign sites in the body. These metastatic cells survive by overriding restrictions that normally constrain cell colonization into dissimilar tissues. For example, typical mammary epithelial cells will generally not grow or survive if transplanted to the lung, yet lung metastases are a major cause of breast cancer morbidity and mortality. Recent evidence suggests that dissemination of metastatic cells through the body can occur long before clinical presentation of the primary tumor. These micrometastatic cells may remain dormant for many months or years following the detection and removal of the primary tumor. Thus, a better understanding of the mechanisms that allow for the growth and survival of metastatic cells in a foreign microenvironment is critical for the improvement of therapeutics designed to fight metastatic cancer and diagnostics for the early detection and localization of metastases.

2.2.3. Cancer Cell Signaling

[008] Cancer is a disease of aberrant signal transduction. Aberrant cell signaling overrides anchorage-dependent constraints on cell growth and survival (Rhim *et al.*, 1997, *Crit. Rev. in Oncogenesis* 8:305; Patarca, 1996, *Crit. Rev. in Oncogenesis* 7:343; Malik *et al.*, 1996, *Biochimica et Biophysica Acta* 1287:73; Cance *et al.*, 1995, *Breast Cancer Res. Treat.* 35:105). Tyrosine kinase activity is induced by extracellular matrix (ECM) anchorage and indeed, the expression or function of tyrosine kinases is usually increased in malignant cells (Rhim *et al.*, 1997, *Critical Reviews in Oncogenesis* 8:305; Cance *et al.*, 1995, *Breast Cancer Res. Treat.* 35:105; Hunter, 1997, *Cell* 88:333). Based on evidence that tyrosine kinase activity is necessary for malignant cell growth, tyrosine kinases have been targeted with new therapeutics (Levitzki *et al.*, 1995, Sc*ience* 267:1782; Kondapaka *et al.*, 1996, *Mol. & Cell. Endocrinol.* 117:53; Fry *et al.*, 1995, *Curr. Opin. in BioTechnology* 6:662). Unfortunately, obstacles associated with specific targeting to tumor cells often limit the application of these drugs. In particular, tyrosine kinase activity is often vital for the function and survival of benign tissues (Levitzki *et al.*, 1995, *Science* 267:1782). To minimize collateral toxicity, it is critical to first identify and then target tyrosine kinases that are selectively overexpressed in tumor cells.

2.2.4. Cancer Therapy

[009] Barriers to the development of anti-metastasis agents have been the assay systems that are used to design and evaluate these drugs. Most conventional cancer therapies target rapidly growing cells. However, cancer cells do not necessarily grow more rapidly but instead survive and grow under conditions that are non-permissive to normal cells (Lawrence and Steeg, 1996, *World J. Urol.* 14:124-130). These fundamental differences between the behavior of normal and malignant cells provide opportunities for therapeutic targeting. The paradigm that micrometastatic tumors have already disseminated throughout the body emphasizes the need to evaluate potential chemotherapeutic drugs in the context of a foreign and three-dimensional microenvironment. Many standard cancer drug assays measure tumor cell growth or survival under typical cell culture conditions (*i.e.*, monolayer growth). However, cell behavior in two-dimensional assays often does not reliably predict tumor cell behavior *in vivo*.

[0010] Currently, cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient (*see*, *e.g.*, Stockdale, 1998, "Principles of Cancer Patient Management," in Scientific American: Medicine, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. IV). Recently, cancer therapy may also involve biological therapy or immunotherapy. All of these approaches can pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of the patient or may be unacceptable to the patient. Additionally, surgery may not completely remove the neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue, and radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent and, although it can be effective, is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of the cancer cells. Biological therapies/immunotherapies are limited in number and each therapy is generally effective for only a very specific type of cancer.

[0011] With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A significant majority of cancer chemotherapeutics act by inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of the deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division (*see*, *e.g.*, Gilman *et al.*, 1990, Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed. (Pergamom Press, New York)). These agents, which include alkylating agents, such as nitrosourea, anti-metabolites, such as methotrexate and hydroxyurea, and other agents, such as etoposides, campathecins, bleomycin, doxorubicin,

daunorubicin, *etc.*, although not necessarily cell cycle specific, kill cells during S phase because of their effect on DNA replication. Other agents, specifically colchicine and the vinca alkaloids, such as vinblastine and vincristine, interfere with microtubule assembly resulting in mitotic arrest. Chemotherapy protocols generally involve administration of a combination of chemotherapeutic agents to increase the efficacy of treatment.

[0012] Despite the availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks (*see, e.g.*, Stockdale, 1998, "Principles Of Cancer Patient Management" in Scientific American Medicine, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. X). Almost all chemotherapeutic agents are toxic, and chemotherapy causes significant, and often dangerous, side effects, including severe nausea, bone marrow depression, immunosuppression, *etc.* Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even those agents that act by mechanisms different from the mechanisms of action of the drugs used in the specific treatment; this phenomenon is termed pleiotropic drug or multidrug resistance. Thus, because of drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

[0013] There is a significant need for alternative cancer treatments, particularly for treatment of cancer that has proved refractory to standard cancer treatments, such as surgery, radiation therapy, chemotherapy, and hormonal therapy. Further, it is uncommon for cancer to be treated by only one method. Thus, there is a need for development of new therapeutic agents for the treatment of cancer and new, more effective, therapy combinations for the treatment of cancer.

2.2.5. Other Hyperproliferative Disorders

2.2.5.1. Asthma

[0014] Asthma is a disorder characterized by intermittent airway obstruction. In western countries, it affects 15% of the pediatric population and 7.5% of the adult population (Strachan et al., 1994, *Arch. Dis. Child* 70:174-178). Most asthma in children and young adults is initiated by IgE mediated allergy (atopy) to inhaled allergens such as house dust mite and cat dander allergens. However, not all asthmatics are atopic, and most atopic individuals do not have asthma. Thus, factors in addition to atopy are necessary to induce the disorder (Fraser *et al.*, eds.,1994, Synopsis of Diseases of the Chest: 635-53 (WB Saunders Company, Philadelphia); Djukanovic *et al.*, 1990, *Am. Rev. Respir. Dis.*

142:434-457). Asthma is strongly familial, and is due to the interaction between genetic and environmental factors. The genetic factors are thought to be variants of normal genes ("polymorphisms") which alter their function to predispose to asthma.

[0015] Asthma may be identified by recurrent wheeze and intermittent air flow limitation. An asthmatic tendency may be quantified by the measurement of bronchial hyper-responsiveness in which an individual's dose-response curve to a broncho-constrictor such as histamine or methacholine is constructed. The curve is commonly summarized by the dose which results in a 20% fall in air flow (PD20) or the slope of the curve between the initial air flow measurement and the last dose given (slope).

[0016] In the atopic response, IgE is produced by B-cells in response to allergen stimulation. These antibodies coat mast cells by binding to the high affinity receptor for IgE and initiate a series of cellular events leading to the destabilization of the cell membrane and release of inflammatory mediators. This results in mucosal inflammation, wheezing, coughing, sneezing and nasal blockage.

[0017] Atopy can be diagnosed by (i) a positive skin prick test in response to a common allergen; (ii) detecting the presence of specific serum IgE for allergen; or (iii) by detecting elevation of total serum IgE.

2.2.5.2. COPD

[0018] Chronic obstructive pulmonary disease (COPD) is an umbrella term frequently used to describe two conditions of fixed airways disorders, chronic bronchitis and emphysema. Chronic bronchitis and emphysema are most commonly caused by smoking; approximately 90% of patients with COPD are or were smokers. Although approximately 50% of smokers develop chronic bronchitis, only 15% of smokers develop disabling airflow obstruction. Certain animals, particularly horses, suffer from COPD as well.

[0019] The airflow obstruction associated with COPD is progressive, may be accompanied by airway hyperactivity, and may be partially reversible. Non-specific airway hyper-responsiveness may also play a role in the development of COPD and may be predictive of an accelerated rate of decline in lung function.

[0020] COPD is a significant cause of death and disability. It is currently the fourth leading cause of death in the United States and Europe. Treatment guidelines advocate early detection and implementation of smoking cessation programs to help reduce morbidity and mortality due to the disorder. However, early detection and diagnosis has been difficult for a number of reasons. COPD takes years to develop and acute episodes of bronchitis often are not recognized by the general practitioner as early signs of COPD. Many patients

exhibit features of more than one disorder (*e.g.*, chronic bronchitis or asthmatic bronchitis) making precise diagnosis a challenge, particularly early in the etiology of the disorder. Also, many patients do not seek medical help until they are experiencing more severe symptoms associated with reduced lung function, such as dyspnea, persistent cough, and sputum production. As a consequence, the vast majority of patients are not diagnosed or treated until they are in a more advanced stage of the disorder.

2.2.5.3. Mucin

[0021] Mucins are a family of glycoproteins secreted by the epithelial cells including those at the respiratory, gastrointestinal and female reproductive tracts. Mucins are responsible for the viscoelastic properties of mucus (Thornton *et al.*, 1997, *J. Biol. Chem.* 272:9561-9566). Nine mucin genes are known to be expressed in man: MUC 1, MUC 2, MUC 3, MUC 4, MUC 5AC, MUC 5B, MUC 6, MUC 7 and MUC 8 (Bobek *et al.*, 1993, *J. Biol. Chem.* 268:20563-9; Dusseyn *et al.*, 1997, *J. Biol. Chem.* 272:3168-78; Gendler *et al.*, 1991, *Am. Rev.Resp. Dis.* 144:S42-S47; Gum *et al.*, 1989, *J. Biol. Chem.* 264:6480-6487; Gum *et al.*, 1990, *Biochem. Biophys. Res. Comm.* 171:407-415; Lesuffleur *et al.*, 1995, J. *Biol. Chem.* 270:13665-13673; Meerzaman *et al.*, 1994, *J. Biol. Chem.* 269:12932-12939; Porchet *et al.*, 1991, *Biochem. Biophys. Res. Comm.* 175:414-422; Shankar *et al.*, 1994, *Biochem. J.* 300:295-298; Toribara *et al.*, 1997, J. *Biol. Chem.* 272:16398-403). Many airway disorders such chronic bronchitis, chronic obstructive pulmonary disease, bronchietactis, asthma, cystic fibrosis and bacterial infections are characterized by mucin overproduction (Prescott *et al.*, *Eur. Respir. J.*, 1995, 8:1333-1338; Kim *et al.*, *Eur. Respir. J.*, 1997, 10:1438; Steiger *et al.*, 1995, *Am. J. Respir. Cell Mol. Biol.*, 12:307-314). Mucociliary impairment caused by mucin hypersecretion leads to airway mucus plugging which promotes chronic infection, airflow obstruction and sometimes death. For example, COPD, a disorder characterized by slowly progressive and irreversible airflow limitation, is a major cause of death in developed countries. The respiratory degradation consists mainly of decreased luminal diameters due to airway wall thickening and increased mucus caused by goblet cell hyperplasia and hypersecretion. Epidermal growth factor (EGF) is known to upregulate epithelial cell proliferation, and mucin production/secretion (Takeyama *et al.*, 1999, *Proc. Natl. Acad. Sci. USA* 96:3081-6; Burgel *et al.*, 2001, *J. Immunol.* 167:5948-54). EGF also causes mucin-secreting cells, such as goblet cells, to proliferate and increase mucin production in airway epithelia (Lee *et al.*, 2000, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 278:185-92; Takeyama *et al.*, 2001, *Am. J. Respir. Crit. Care. Med.* 163:511-6; Burgel *et al.*, 2000, *J. Allergy Clin. Immunol.* 106:705-12). Historically, mucus hypersecretion has been treated in two ways: physical methods to

increase clearance and mucolytic agents. Neither approach has yielded significant benefit to the patient or reduced mucus obstruction. Therefore, it would be desirable to have methods for reducing mucin production and treating the disorders associated with mucin hypersecretion.

2.2.5.4. Restenosis

[0022] Vascular interventions, including angioplasty, stenting, atherectomy and grafting are often complicated by undesirable effects. Exposure to a medical device which is implanted or inserted into the body of a patient can cause the body tissue to exhibit adverse physiological reactions. For instance, the insertion or implantation of certain catheters or stents can lead to the formation of emboli or clots in blood vessels. Other adverse reactions to vascular intervention include endothelial cell proliferation which can lead to hyperplasia, restenosis, *i.e.* the re-occlusion of the artery, occlusion of blood vessels, platelet aggregation, and calcification. Treatment of restenosis often involves a second angioplasty or bypass surgery. In particular, restenosis may be due to endothelial cell injury caused by the vascular intervention in treating a restenosis.

[0023] Angioplasty involves insertion of a balloon catheter into an artery at the site of a partially obstructive atherosclerotic lesion. Inflation of the balloon is intended to rupture the intima and dilate the obstruction. About 20 to 30% of obstructions reocclude in just a few days or weeks (Eltchaninoff *et al.*, 1998, *J. Am Coll. Cardiol.* 32: 980-984). Use of stents reduces the re-occlusion rate, however a significant percentage continues to result in restenosis. The rate of restenosis after angioplasty is dependent upon a number of factors including the length of the plaque. Stenosis rates vary from 10% to 35% depending the risk factors present. Further, repeat angiography one year later reveals an apparently normal lumen in only about 30% of vessels having undergone the procedure.

[0024] Restenosis is caused by an accumulation of extracellular matrix containing collagen and proteoglycans in association with smooth muscle cells which is found in both the atheroma and the arterial hyperplastic lesion after balloon injury or clinical angioplasty. Some of the delay in luminal narrowing with respect to smooth muscle cell proliferation may result from the continuing elaboration of matrix materials by neointimal smooth muscle cells. Various mediators may alter matrix synthesis by smooth muscle cells *in vivo*.

2.2.5.5. Neointimal Hyperplasia

[0025] Neointimal hyperplasia is the pathological process that underlies graft atherosclerosis, stenosis, and the majority of vascular graft occlusion. Neointimal hyperplasia is commonly seen after various forms of vascular injury and a major component

of the vein graft's response to harvest and surgical implantation into high-pressure arterial circulation.

[0026] Smooth muscle cells in the middle layer (*i.e.* media layer) of the vessel wall become activated, divide, proliferate and migrate into the inner layer (*i.e.* intima layer). The resulting abnormal neointimal cells express pro-inflammatory molecules, including cytokines, chemokines and adhesion molecules that further trigger a cascade of events that lead to occlusive neointimal disease and eventually graft failure.

[0027] The proliferation of smooth muscle cells is a critical event in the neointimal hyperplastic response. Using a variety of approaches, studies have clearly demonstrated that blockade of smooth muscle cell proliferation resulted in preservation of normal vessel phenotype and function, causing the reduction of neointimal hyperplasia and graft failure.

[0028] Existing treatments for the indications discussed above is inadequate; thus, there exists a need for improved treatments for the above indications.

2.3. EphA2

[0029] EphA2 is a 130 kDa receptor tyrosine kinase that is expressed in adult epithelia, where it is found at low levels and is enriched within sites of cell-cell adhesion (Zantek *et al*, 1999, *Cell Growth & Differentiation* 10:629; Lindberg *et al.*, 1990, *Molecular & Cellular Biology* 10:6316). This subcellular localization is important because EphA2 binds ligands (known as EphrinsA1 to A5) that are anchored to the cell membrane (Eph Nomenclature Committee, 1997, *Cell* 90:403; Gale *et al.*, 1997, *Cell & Tissue Research* 290: 227). The primary consequence of ligand binding is EphA2 autophosphorylation (Lindberg *et al.*, 1990, *supra*). However, unlike other receptor tyrosine kinases, EphA2 retains enzymatic activity in the absence of ligand binding or phosphotyrosine content (Zantek *et al.*, 1999, *supra*). EphA2 is upregulated on a large number hyperproliferating cells, including aggressive carcinoma cells.

3. SUMMARY OF THE INVENTION

[0030] EphA2 is overexpressed and functionally altered in a large number of malignant carcinomas. EphA2 is an oncoprotein and is sufficient to confer metastatic potential to cancer cells. EphA2 is also associated with other hyperproliferating cells and is implicated in diseases caused by cell hyperproliferation. The present invention stems from the inventors' discovery that administration of *Listeria* that express an EphA2 antigenic peptide to a subject provides beneficial therapeutic and prophylactic benefits against hyperproliferative disorders involving EphA2 overexpressing cells. Without being bound by any mechanism or theory, it is believed that the therapeutic and prophylactic benefit is

the result of an immune response elicited by administration of the EphA2 antigenic peptide-expressing *Listeria*.

[0031] The present invention thus provides *Listeria*-based EphA2 vaccines and methods for their use. The *Listeria*-based EphA2 vaccines of the present invention can elicit a cellular immune response, a humoral immune response, or both. Where the immune response is a cellular immune response, it can be a Tc, Th1 or a Th2 immune response. In a preferred embodiment, the immune response is a Th2 cellular immune response.

[0032] In a preferred embodiment, a *Listeria*-based EphA2 vaccine of the invention expresses one or more epitopes of EphA2 that is selectively exposed or increased on cancer cells relative to non-cancer cells (*i.e.*, normal, healthy cells or cells that are not hyperproliferative). In one embodiment, the cancer is of an epithelial cell origin. In other embodiments, the cancer is a cancer of the skin, lung, colon, prostate, breast, ovary, esophageal, bladder, or pancreas or is a renal cell carcinoma or a melanoma. In another embodiment, the cancer is of a T cell origin. In yet other embodiments, the cancer is a leukemia or a lymphoma.

[0033] In a preferred embodiment, the methods and compositions of the invention are used to prevent, treat or manage EphA2-expressing tumor metastases. In a preferred embodiment, the EphA2-expressing cells against which an immune response is sought ("target cells") overexpress EphA2 relative to a normal healthy cell of the same type as assessed by an assay described herein or known to one of skill in the art (e.g., an immunoassay such as an ELISA or a Western blot, a Northern blot or RT-PCR). In a preferred embodiment, less EphA2 on the target cells is bound to ligand compared to a normal, healthy cell of the same type, either as a result of decreased cell-cell contacts, altered subcellular localization, or increases in amount of EphA2 relative to ligand. In another embodiment, approximately 10% or less, approximately 15% or less, approximately 20% or less, approximately 25% or less, approximately 30% or less, approximately 35% or less, approximately 40% or less, approximately 45% or less, approximately 50% or less, approximately 55% or less, approximately 60% or less, approximately 65% or less, approximately 70% or less, approximately 75% or less, approximately 80% or less, approximately 85% or less, approximately 90% or less, or approximately 95% or less of EphA2 on the target cells is bound to ligand (*e.g.*, EphrinA1) compared to a normal, healthy cell of the same type as assessed by an assay known in the art (*e.g.*, an immunoassay). In another embodiment, 1-10 fold, 1-8 fold, 1-5 fold, 1-4 fold or 1-2 fold, or 1 fold, 1.5 fold, 2 fold, 3 fold, 4 fold, 5 fold, or 10 fold less EphA2 on target cells is bound to ligand (*e.g.*,

EphrinA1) compared to a normal, healthy cell of the same type as assessed by an assay known in the art (*e.g.*, an immunoassay).

[0034] Thus, the present invention provides methods of eliciting an immune response against an EphA2-expressing cell, said method comprising administering to an individual a *Listeria*-based EphA2 vaccine in an amount effective to elicit an immune response against an EphA2-expressing cell.

[0035] The present invention provides a method of treating, preventing or managing a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to an individual a *Listeria*-based EphA2 vaccine in an amount effective treat or prevent the hyperproliferative disorder (e.g., a neoplastic hyperproliferative disorder and a non-neoplastic hyperproliferative disorder). The present invention also provides *Listeria*-based EphA2 vaccines useful for eliciting an immune response against an EphA2-expressing cell and/or for treating, preventing or managing a hyperproliferative disorder of EphA2-expressing cells.

[0036] The *Listeria*-based EphA2 vaccines may comprise *Listeria* as an EphA2 antigenic peptide expression vehicle. In a preferred embodiment, the *Listeria* bacteria administered to a subject (preferably, a human subject) as an EphA2 antigenic expression vehicle are attenuated. For example, the attenuated *Listeria* bacteria administered to a subject (preferably, a human subject) maybe attenuated in their tissue tropism (*e.g.*, inlB mutant) or ability to spread from cell to cell (*e.g.*, actA mutant). In a specific embodiment, the attenuated *Listeria* bacteria administered to a subject (preferably, a human subject) as an EphA2 antigenic expression vehicle comprise a mutation (*e.g.*, a deletion, addition or substitution) in one or more internalins (e.g., inlA and/or inlB) and such mutation results in or contributes to the attenuation of the *Listeria*. In another embodiment, the attenuated *Listeria* bacteria administered to a subject (preferably, a human subject) as an EphA2 antigenic expression vehicle are attenuated in their tissue tropism (*e.g.*, inlB mutant) and in their ability to spread from cell to cell (*e.g.*, actA mutant). In a preferred embodiment, the attenuated *Listeria* bacteria administered to subject (preferably, a human subject) as an EphA2 antigenic expression vehicle comprise a mutation (*e.g.*, a deletion, addition or substitution) in internalin B and a mutation in actA, and such mutations result in or contribute to the attenuation of the *Listeria*.

[0037] The *Listeria* (preferably, the attenuated *Listeria*) of the invention are preferably engineered to express an EphA2 antigenic peptide that is secreted from the *Listeria*. In a specific embodiment, a nucleic acid encoding an EphA2 antigenic peptide comprises a nucleotide sequence encoding a secretory signal, *e.g.*, the SecA secretory signal

or Tat signal, operatively linked to the nucleotide sequence encoding the EphA2 antigenic peptide. In some embodiments, the signal sequence is a *Listeria* signal sequence. In other embodiments, the signal sequence is a bacterial signal sequence other than a *Listeria* signal sequence (*i.e.*, a non-*Listeria* bacterial signal sequence).

[0038] Strains of *Listeria* bacteria suitable for use in the methods and compositions of the invention include, but are not limited to, *Listeria grayi*, *Listeria innocua*, *Listeria ivanovii*, *Listeria monocytogenes*, *Listeria seeligeri* and *Listeria welshimeri*. A preferred strain of *Listeria* bacteria for use in the methods and compositions of the invention is *Listeria monocytogenes*.

[0039] The compositions and methods of the present invention are useful in the treatment, prevention and/or management of hyperproliferative diseases. In certain embodiments, the hyperproliferative disease is cancer. In certain embodiments, the cancer is of an epithelial cell origin and/or involves cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells. In specific embodiments, the cancer is a cancer of the skin, lung, colon, breast, ovary, esophageal, prostate, bladder or pancreas or is a renal cell carcinoma or melanoma. In yet other embodiments, the cancer is of a T cell origin. In specific embodiments, the cancer is a leukemia or a lymphoma. In yet other embodiments, the hyperproliferative disorder is non-neoplastic. In specific embodiments, the non-neoplastic hyperproliferative disorder is an epithelial cell disorder. Exemplary non-neoplastic hyperproliferative disorders are asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis. In certain embodiments, the hyperproliferative disease is an endothelial cell disorder.

[0040] The EphA2 antigenic peptide for use in accordance with the methods and compositions of the present invention may comprise full length EphA2 or an antigenic fragment, analog or derivative thereof. In certain embodiments, the EphA2 antigenic peptide comprises the extracellular domain of EphA2 or the intracellular domain of EphA2. In certain embodiments, the EphA2 antigenic peptide lacks the EphA2 transmembrane domain. In certain embodiments, the EphA2 antigenic peptide comprises the EphA2 extracellular and intracellular domains and lacks the transmembrane domain of EphA2. In certain embodiments, the EphA2 antigenic peptide comprises full length EphA2 or a fragment thereof with a substitution of lysine to methionine at amino acid residue 646 of EphA2. In certain embodiments, the EphA2 antigenic peptide comprises the extracellular and intracellular domains of EphA2, lacks the transmembrane domain of EphA2 and has a substitution of lysine to methionine at amino acid residue 646 of EphA2. In certain

embodiments the EphA2 antigenic peptide is a chimeric polypeptide comprising at least an antigenic portion of EphA2 and a second polypeptide.

[0041] An EphA2 antigenic peptide-expressing *Listeria* may express one or a plurality of EphA2 antigenic peptides. In a specific embodiment, an EphA2 antigenic peptide-expressing *Listeria* expresses 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more EphA2 antigenic peptides, or 2-5, 2-10, 2-20, 10-20, or 15-25 EphA2 antigenic peptides. The plurality of EphA2 antigenic peptides may be expressed from a single expression construct or a plurality of expression constructs. The expression construct(s) can be episomal or integrated into the *Listeria* genome. For example, in certain embodiments, the genome of the *Listeria* vaccine strain comprises one or more gene expression cassettes, which in combination encode both the intracellular and extracellular domains of EphA2. In specific embodiments, the one or more expression cassettes are integrated into the *Listeria* genome.

[0042] A vaccine of the invention may have one or a plurality of EphA2 antigenic peptide-expressing *Listeria*. In a specific embodiment, a vaccine of the invention has 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more EphA2 antigenic peptide-expressing *Listeria*, or 2-5, 2-10, 2-20, 10-20, or 15-25 EphA2 antigenic peptide-expressing *Listeria*.

[0043] The methods of the present invention encompass combination therapy with a *Listeria*-based EphA2 vaccine and one or more additional therapies, for example an additional anti-cancer therapy. In certain embodiments, the additional anti-cancer therapy is an agonistic EphA2 antibody, *i.e.*, antibody that binds to EphA2 and induces signaling and phosphorylation of EphA2. In other embodiments, the additional anti-cancer therapy is an anti-idiotype of an anti-EphA2 antibody. In yet other embodiments, the additional anti-cancer therapy is chemotherapy, biological therapy, immunotherapy, radiation therapy, hormonal therapy, or surgery.

[0044] In certain aspects of the present invention, the *Listeria*-based vaccines of the invention are administered in combination with a therapy that increases EphA2 internalization. In specific embodiments, the agent is an EphA2 agonist, for example an antibody, peptide (*see*, *e.g.*, Koolpe *et al.*, 2002, J. Biol. Chem. 277(49):46974-46979) or small molecule. In other specific embodiments, the agent is an inhibitor of a phosphatase that modulates EphA2, *e.g.*, low molecular weight tyrosine phosphatase (LMW-PTP).

[0045] The vaccines of the invention can be administered, for example, by mucosal, intranasal, parenteral, intramuscular, intravenous, oral or intraperitoneal routes. In a specific embodiment, the vaccines of the invention are administered locally to the site of a disease, by, *e.g.*, implantation or intratumoral injection.

[0046] In other embodiments, the *Listeria*-based EphA2 vaccines of the invention are used to treat, prevent and/or manage a non-cancer disease or disorder associated with cell hyperproliferation, such as but not limited to asthma, chronic obstructive pulmonary disease, restenosis (smooth muscle and/or endothelial), psoriasis, *etc*. In preferred embodiments, the hyperproliferative cells are epithelial. In preferred embodiments, the hyperproliferative cells overexpress EphA2. In another preferred embodiment, some (e.g., 5% or less, 10% or less, 15% or less, 20% or less, 25% or less, 30% or less, 35% or less, 40% or less, 45% or less, 50% or less, 55% or less, 60% or less, 75% or less, 85% or less) EphA2 is not bound to ligand as assessed by an assay known in the art (*e.g.*, an immunoassay), either as a result of decreased cell-cell contacts, altered subcellular localization, or increases in the amount of EphA2 relative to EphA2-ligand.

[0047] In yet other aspects of the invention, the *Listeria*-based EphA2 vaccines are used to treat, prevent and/or manage a disorder associated with or involving aberrant angiogenesis. The *Listeria*-based EphA2 vaccines are used to elicit an immune response against EphA2 expressed on neovasculature. Thus, the present invention provides methods of treating, preventing and/or managing a disorder associated with or involving aberrant angiogenesis comprising administering to a subject in need thereof a composition comprising an EphA2 antigenic peptide-expressing *Listeria* bacterium in an amount effective to treat, prevent and/or manage a disorder associated with or involving aberrant angiogenesis. Examples of such diseases include, but are not limited to, macular degeneration, diabetic retinopathy, retinopathy of prematurity, vascular restenosis, infantile hemangioma, verruca vulgaris, psoriasis, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa, rheumatoid arthritis, ankylosing spondylitis, systemic lupus, psoriatic arthropathy, Reiter's syndrome, and Sjogren's syndrome, endometriosis, preeclampsia, atherosclerosis and coronary artery disease.

[0048] The methods and compositions of the invention are useful not only in untreated patients but are also useful in the treatment of patients partially or completely refractory to current standard and experimental cancer therapies, including but not limited to chemotherapies, hormonal therapies, biological therapies, radiation therapies, and/or surgery as well as to improve the efficacy of such treatments. In particular, EphA2 expression has been implicated in increasing levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy. In addition, EphA2 overexpression can override the need for estrogen receptor activity thus contributing to tamoxifen resistance in breast cancer cells. Accordingly, in a preferred embodiment, the invention provides

therapeutic and prophylactic methods for the treatment, prevention or management of cancer that has been shown to be or may be refractory or non-responsive to therapies other than those comprising administration of *Listeria*-based EphA2 vaccines of the invention. In a specific embodiment, one or more *Listeria*-based EphA2 vaccines of the invention are administered to a patient refractory or non-responsive to a non-EphA2-based treatment, particularly tamoxifen treatment or a treatment in which resistance is associated with increased IL-6 levels, to render the patient non-refractory or responsive. The treatment to which the patient had previously been refractory or non-responsive can then be administered with therapeutic effect.

[0049] The methods and compositions of the invention are useful not only in untreated patients but are also useful in the treatment of patients partially or completely refractory to current standard and experimental therapies for non-neoplastic hyperproliferative disorders and/or disorders associated with or involving aberrant angiogenesis. The methods and compositions of the invention are useful for the treatment of patients partially or completely refractory to current standard and experimental therapies for neoplastic hyperproliferative disorders and/or disorders associated with or involving aberrant angiogenesis (*e.g.*, macular degeneration, diabetic retinopathy, retinopathy of prematurity, vascular restenosis, infantile hemangioma, verruca vulgaris, psoriasis, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa, rheumatoid arthritis, ankylosing spondylitis, systemic lupus, psoriatic arthropathy, Reiter's syndrome, and Sjogren's syndrome, endometriosis, preeclampsia, atherosclerosis and coronary artery disease), asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis).

[0050] The present invention also provides kits comprising the vaccines or vaccine components of the invention.

3.1. DEFINITIONS

[0051] As used herein, the term "*Listeria*-based EphA2 vaccine" refers to a *Liseria* bacterium that has been engineered to express an EphA2 antigenic peptide, or a composition comprising such a bacterium. The *Listeria*-based EphA2 vaccines of the invention, when administered in an effective amount, elicit an immune response against EphA2 on hyperproliferative cells. Strains of *Listeria* bacteria suitable for use in a vaccine of the invention include, but are not limited to, *Listeria grayi, Listeria innocua, Listeria ivanovii, Listeria monocytogenes, Listeria seeligeri* and *Listeria welshimeri*. In a preferred embodiment, the *Listeria* is *Listeria monocytogenes*.

[0052] As used herein, the terms "EphA2 antigenic peptide" and "EphA2 antigenic polypeptide" refer to an EphA2 polypeptide, preferably of SEQ ID NO:2, or a fragment, analog or derivative thereof comprising one or more B cell epitopes or T cell epitopes of EphA2. The EphA2 polypeptide may be from any species. In certain embodiments, an EphA2 polypeptide refers to the mature, processed form of EphA2. In other embodiments, an EphA2 polypeptide refers to an immature form of EphA2.

[0053] The nucleotide and/or amino acid sequences of EphA2 polypeptides can be found in the literature or public databases, or the nucleotide and/or amino acid sequences can be determined using cloning and sequencing techniques known to one of skill in the art. For example, the nucleotide sequence of human EphA2 can be found in the GenBank database (see, *e.g.*, Accession Nos. BC037166, M59371 and M36395). The amino acid sequence of human EphA2 can be found in the GenBank database (see, *e.g.*, Accession Nos. NP_004422, AAH37166 and AAA53375). Additional non-limiting examples of amino acid sequences of EphA2 are listed in Table 1, *infra*.

[0054] Table 1

Species	**GenBank Accession No.**
Mouse	NP_034269, AAH06954
Rat	XP_345597
Chicken	BAB63910

[0055] In certain embodiments, the EphA2 antigenic peptides are not one or more of the following peptides: TLADFDPRV (SEQ ID NO:3); VLLLVLAGV (SEQ ID NO:4); VLAGVGFFI (SEQ ID NO:5); IMNDMPIYM (SEQ ID NO:6); SLLGLKDQV (SEQ ID NO:7); WLVPIGQCL (SEQ ID NO:8); LLWGCALAA (SEQ ID NO:9); GLTRTSVTV (SEQ ID NO:10); NLYYAESDL (SEQ ID NO:11); KLNVEERSV (SEQ ID NO:12); IMGQFSHHN (SEQ ID NO:13); YSVCNVMSG (SEQ ID NO:14); MQNIMNDMP (SEQ ID NO:15); EAGIMGQFSHHNIIR (SEQ ID NO:16); PIYMYSVCNVMSG (SEQ ID NO:17); DLMQNIMNDMPIYMYS (SEQ ID NO:18). In certain specific embodiments, the EphA2 antigenic peptide is not any of SEQ ID NO:3-12, is not SEQ ID NO:13-15, and/or is not SEQ ID NO:16-18. In yet another specific enbodiment, the EphA2 antigenic peptide is not SEQ ID NO:3-18.

[0056] As used herein, the term "analog" in the context of a proteinaceous agent (*e.g.*, a peptide, polypeptide, protein or antibody) refers to a proteinaceous agent that possesses a similar or identical function as a second proteinaceous agent (*e.g.*, an EphA2 polypeptide) but does not necessarily comprise a similar or identical amino acid sequence or structure of the second proteinaceous agent. A proteinaceous agent that has a similar amino

acid sequence refers to a proteinaceous agent that satisfies at least one of the following: (a) a proteinaceous agent having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a second proteinaceous agent; (b) a proteinaceous agent encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding a second proteinaceous agent of at least 20 amino acid residues, at least 30 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, or at least 150 amino acid residues; and (c) a proteinaceous agent encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence encoding a second proteinaceous agent. A proteinaceous agent with similar structure to a second proteinaceous agent refers to a proteinaceous agent that has a similar secondary, tertiary or quaternary structure of the second proteinaceous agent. The structure of a proteinaceous agent can be determined by methods known to those skilled in the art, including but not limited to, X-ray crystallography, nuclear magnetic resonance, and crystallographic electron microscopy. Preferably, the proteinaceous agent has EphA2 activity.

[0057] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

[0058] The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of

Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87: 2264-2268, modified as in Karlin and Altschul, 1993 , *Proc. Natl. Acad. Sci. U.S.A.* 90: 5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, *J. Mol. Biol.* 215: 403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score-50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25: 3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, the NCBI website). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *CABIOS* 4: 11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[0059] The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

[0060] As used herein, the term “analog” in the context of a non-proteinaceous analog refers to a second organic or inorganic molecule which possesses a similar or identical function as a first organic or inorganic molecule and is structurally similar to the first organic or inorganic molecule.

[0061] As used herein, the terms “attenuated” and “attenuation” refer to a modification(s) so that the *Listeria* are less pathogenic. The end result of attenuation is that the risk of toxicity as well as other side effects is decreased when the *Listeria* are administered to a subject.

[0062] As used herein, the term “derivative” in the context of a proteinaceous agent (*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to a proteinaceous agent that comprises the amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions, and/or additions. The term “derivative” as used herein

also refers to a proteinaceous agent which has been modified, *i.e.*, by the covalent attachment of a type of molecule to the proteinaceous agent. For example, but not by way of limitation, a derivative of a proteinaceous agent may be produced, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a proteinaceous agent may also be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a proteinaceous agent may contain one or more non-classical amino acids. A derivative of a proteinaceous agent possesses an identical function(s) as the proteinaceous agent from which it was derived.

[0063] As used herein, the term "derivative" in the context of EphA2 proteinaceous agents refers to a proteinaceous agent that comprises an amino acid sequence of an EphA2 polypeptide or a fragment of an EphA2 polypeptide that has been altered by the introduction of amino acid residue substitutions, deletions or additions (*i.e.,* mutations). The term "derivative" as used herein in the context of EphA2 proteinaceous agents also refers to an EphA2 polypeptide or a fragment of an EphA2 polypeptide which has been modified, *i.e*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, an EphA2 polypeptide or a fragment of an EphA2 polypeptide may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, *etc.* A derivative of an EphA2 polypeptide or a fragment of an EphA2 polypeptide may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc*. Further, a derivative of an EphA2 polypeptide or a fragment of an EphA2 polypeptide may contain one or more non-classical amino acids. In one embodiment, a polypeptide derivative possesses a similar or identical function as an EphA2 polypeptide or a fragment of an EphA2 polypeptide described herein. In another embodiment, a derivative of EphA2 polypeptide or a fragment of an EphA2 polypeptide has an altered activity when compared to an unaltered polypeptide. For example, a derivative of an EphA2 polypeptide or fragment thereof can differ in phosphorylation relative to an EphA2 polypeptide or fragment thereof.

[0064] As used herein, the term "derivative" in the context of a non-proteinaceous agent refers to a second organic or inorganic molecule that is formed based upon the

structure of a first organic or inorganic molecule. A derivative of an organic molecule includes, but is not limited to, a molecule modified, *e.g.*, by the addition or deletion of a hydroxyl, methyl, ethyl, carboxyl, nitryl, or amine group. An organic molecule may also, for example, be esterified, alkylated and/or phosphorylated.

[0065] As used herein, the term "EphrinA1 polypeptide" refers to EphrinA1, an analog, derivative or a fragment thereof, or a fusion protein comprising EphrinA1, an analog, derivative or a fragment thereof. The EphrinA1 polypeptide may be from any species. In certain embodiments, the term "EphrinA1 polypeptide" refers to the mature, processed form of EphrinA1. In other embodiments, the term "EphrinA1 polypeptide" refers to an immature form of EphrinA1. In accordance with this embodiment, the antibodies of the invention immunospecifically bind to the portion of the immature form of EphrinA1 that corresponds to the mature, processed form of EphrinA1.

[0066] The nucleotide and/or amino acid sequences of EphrinA1 polypeptides can be found in the literature or public databases, or the nucleotide and/or amino acid sequences can be determined using cloning and sequencing techniques known to one of skill in the art. For example, the nucleotide sequence of human EphrinA1 can be found in the GenBank database (see, *e.g.*, Accession No. BC032698). The amino acid sequence of human EphrinA1 can be found in the GenBank database (see, *e.g.*, Accession No. AAH32698). Additional non-limiting examples of amino acid sequences of EphrinA1 are listed in Table 2, *infra*.

Table 2

Species	**GenBank Accession No.**
Mouse	NP_034237
Rat	NP_446051

[0067] In a specific embodiment, a EphrinA1 polypeptide is EphrinA1 from any species. In a preferred embodiment, an EphrinA1 polypeptide is human EphrinA1.

[0068] As used herein, the term "effective amount" refers to the amount of a therapy (*e.g.*, a prophylactic or therapeutic agent) which is sufficient to reduce and/or ameliorate the severity and/or duration of a disorder (*e.g.*, cancer, a non-neoplastic hyperproliferative cell disorder or a disorder associated with aberrant angiogenesis) or a symptom thereof, prevent the advancement of said disorder, cause regression of said disorder, prevent the recurrence, development, or onset of one or more symptoms associated with said disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (*e.g.*, prophylactic or therapeutic agent).

[0069] As used herein, the term "B cell epitope" refers to a portion of an EphA2 polypeptide having antigenic or immunogenic activity in an animal, preferably in a mammal, and most preferably in a mouse or a human. An epitope having immunogenic activity is a portion of an EphA2 polypeptide that elicits an antibody response in an animal. An epitope having antigenic activity is a portion of an EphA2 polypeptide to which an antibody immunospecifically binds as determined by any method well known in the art, for example, by immunoassays. Antigenic epitopes need not necessarily be immunogenic.

[0070] As used herein, the term "T cell epitope" refers to at least a portion of an EphA2 polypeptide, preferably an EphA2 polypeptide of SEQ ID NO:2, that is recognized by a T cell receptor. The term "T cell epitope" encompasses helper T cell (Th) epitopes and cytotoxic T cell (Tc) epitopes. The term "helper T cell epitopes" encompasses Th1 and Th2 epitopes.

[0071] As used herein, the term "fragments" in the context of EphA2 polypeptides include an EphA2 antigenic peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues of the amino acid sequence of an EphA2 polypeptide.

[0072] As used herein, the term "fusion protein" refers to a polypeptide or protein that comprises the amino acid sequence of a first polypeptide or protein or fragment, analog or derivative thereof, and the amino acid sequence of a heterologous polypeptide or protein. In one embodiment, a fusion protein comprises a prophylactic or therapeutic agent fused to a heterologous protein, polypeptide or peptide. In accordance with this embodiment, the heterologous protein, polypeptide or peptide may or may not be a different type of prophylactic or therapeutic agent. For example, two different proteins, polypeptides, or peptides with immunomodulatory activity may be fused together to form a fusion protein. In a preferred embodiment, fusion proteins retain or have improved activity relative to the activity of the original polypeptide or protein prior to being fused to a heterologous protein, polypeptide, or peptide.

[0073] As used herein, the term "heterologous," in the context of a nucleic acid sequence (*e.g.*, a gene) or an amino acid sequence (*e.g.*, a peptide, polypeptide or protein) refers a nucleic acid sequence or an amino acid sequence that is not found in nature to be associated with a second nucleic acid sequence or a second amino acid sequence (*e.g.*, a nucleic acid sequence or an amino acid sequence derived from a different species).

[0074] As used herein, the terms "hyperproliferative cell disorder," "hyperproliferative cell disease," "hyperproliferative disorder," and "hyperproliferative disease" and analogous terms refer to a disorder in which cellular hyperproliferation or any form of excessive cell accumulation causes or contributes to the pathological state or symptoms of the disorder. In some embodiments, the hyperproliferative cell disorder is characterized by hyperproliferating epithelial cells. In other embodiments, the hyperproliferative cell disorder is characterized by hyperproliferating endothelial cells. In other embodiments, the hyperproliferative cell disorder is characterized by hyperproliferating fibroblasts. In certain embodiments, the hyperproliferative cell disorder is not neoplastic. Exemplary non-neoplastic hyperproliferative cell disorders are asthma, chronic pulmonary obstructive disease, fibrosis (*e.g.*, lung, liver, and kidney fibrosis), bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis. In a preferred embodiment, the hyperproliferative cell disorder is characterized by hyperproliferating cells that express (preferably, overexpress) EphA2.

[0075] As used herein, the term "immunospecifically binds to EphA2" and analogous terms refers to peptides, polypeptides, proteins, fusion proteins, and antibodies or fragments thereof that specifically bind to an EphA2 receptor or one or more fragments thereof and do not specifically bind to other receptors or fragments thereof. The terms "immunospecifically binds to EphrinA1" and analogous terms refer to peptides, polypeptides, proteins, fusion proteins, and antibodies or fragments thereof that specifically bind to EphrinA1 or one or more fragments thereof and do not specifically bind to other ligands or fragments thereof. A peptide, polypeptide, protein, or antibody that immunospecifically binds to EphA2 or EphrinA1, or fragments thereof, may bind to other peptides, polypeptides, or proteins with lower affinity as determined by, *e.g.*, immunoassays or other assays known in the art to detect binding affinity. Antibodies or fragments that immunospecifically bind to EphA2 or EphrinA1 may be cross-reactive with related antigens. Preferably, antibodies or fragments thereof that immunospecifically bind to EphA2 or EphrinA1 can be identified, for example, by immunoassays or other techniques known to those of skill in the art. An antibody or fragment thereof binds specifically to EphA2 or EphrinA1 when it binds to EphA2 or EphrinA1 with higher affinity than to any

cross-reactive antigen as determined using experimental techniques, such as radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs). See, *e.g.*, Paul, ed., 1989, Fundamental Immunology, 2^{nd} ed., Raven Press, New York at pages 332-336 for a discussion regarding antibody specificity. In a preferred embodiment, an antibody that immunospecifically binds to EphA2 or EphrinA1 does not bind or cross-react with other antigens. In another embodiment, an antibody that binds to EphA2 or EphrinA1 that is a fusion protein specifically binds to the portion of the fusion protein that is EphA2 or EphrinA1.

[0076] Antibodies of the invention include, but are not limited to, synthetic antibodies, monoclonal antibodies, recombinantly produced antibodies, multispecific antibodies (including bi-specific antibodies), human antibodies, humanized antibodies, chimeric antibodies, intrabodies, single-chain Fvs (scFv) (*e.g.*, including monospecific and bi-specific, etc.), Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. In particular, antibodies of the present invention include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen-binding site that immunospecifically binds to an EphA2 antigen or an EphrinA1 antigen (*e.g.*, one or more complementarity determining regions (CDRs) of an anti-EphA2 antibody or of an anti-EphrinA1 antibody). The antibodies of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass of immunoglobulin molecule.

[0077] As used herein, the term "isolated" in the context of an organic or inorganic molecule (whether it be a small or large molecule), other than a proteinaceous agent or a nucleic acid, refers to an organic or inorganic molecule substantially free of a different organic or inorganic molecule. Preferably, an organic or inorganic molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% free of a second, different organic or inorganic molecule. In a preferred embodiment, an organic and/or inorganic molecule is isolated.

[0078] As used herein, the term "isolated" in the context of a proteinaceous agent (*e.g.*, a peptide, polypeptide, fusion protein, or antibody) refers to a proteinaceous agent which is substantially free of cellular material or contaminating proteins from the cell or tissue source from which it is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a proteinaceous agent in which the proteinaceous agent is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a proteinaceous agent that is substantially free of cellular material includes

preparations of a proteinaceous agent having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein, polypeptide, peptide, or antibody (also referred to as a "contaminating protein"). When the proteinaceous agent is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the proteinaceous agent preparation. When the proteinaceous agent is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the proteinaceous agent. Accordingly, such preparations of a proteinaceous agent have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the proteinaceous agent of interest. In a specific embodiment, proteinaceous agents disclosed herein are isolated.

[0079] As used herein, the term "isolated" in the context of nucleic acid molecules refers to a nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, is preferably substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a specific embodiment, nucleic acid molecules are isolated.

[0080] As used herein, the term "disease" and "disorder" are used interchangeably to refer to a condition.

[0081] As used herein, the term "in combination" refers to the use of more than one therapies (*e.g.*, prophylactic and/or therapeutic agents). The use of the term "in combination" does not restrict the order in which therapies (*e.g.*, prophylactic and/or therapeutic agents) are administered to a subject with a hyperproliferative cell disorder, especially cancer. A first therapy (*e.g.*, prophylactic and/or therapeutic agent) can be administered prior to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (*e.g.*, prophylactic and/or therapeutic agent) to a subject which had, has, or is susceptible to a hyperproliferative cell disorder, especially cancer. The therapies (*e.g.*, prophylactic and/or therapeutic agents) are administered to a subject in a sequence and

within a time interval such that the agent of the invention can act together with the other agent to provide an increased benefit than if they were administered otherwise. Any additional therapy (*e.g.*, prophylactic and/or therapeutic agent) can be administered in any order with the other additional therapy (*e.g.*, prophylactic and/or therapeutic agent).

[0082] As used herein, the phrase "low tolerance" refers to a state in which the patient suffers from side effects from treatment so that the patient does not benefit from and/or will not continue therapy because of the adverse effects and/or the harm from the side effects outweighs the benefit of the treatment.

[0083] As used herein, the terms "manage," "managing" and "management" refer to the beneficial effects that a subject derives from administration of a therapy (*e.g.*, prophylactic and/or therapeutic agent), which does not result in a cure of the disease. In certain embodiments, a subject is administered one or more therapies (*e.g.*, prophylactic and/or therapeutic agents) to "manage" a disease so as to prevent the progression or worsening of the disease.

[0084] As used herein, the term "neoplastic" refers to a disease involving cells that have the potential to metastasize to distal sites and exhibit phenotypic traits that differ from those of non-neoplastic cells, for example, formation of colonies in a three-dimensional substrate such as soft agar or the formation of tubular networks or weblike matrices in a three-dimensional basement membrane or extracellular matrix preparation, such as MATRIGEL™. Non-neoplastic cells do not form colonies in soft agar and form distinct sphere-like structures in three-dimensional basement membrane or extracellular matrix preparations. Neoplastic cells acquire a characteristic set of functional capabilities during their development, albeit through various mechanisms. Such capabilities include evading apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion/metastasis, limitless replicative potential, and sustained angiogenesis. Thus, "non-neoplastic" means that the condition, disease, or disorder does not involve cancer cells.

[0085] As used herein, the phrase "non-responsive/refractory" is used to describe patients treated with one or more currently available therapies (*e.g.*, cancer therapies) such as chemotherapy, radiation therapy, surgery, hormonal therapy and/or biological therapy/immunotherapy, particularly a standard therapeutic regimen for the particular cancer, wherein the therapy is not clinically adequate to treat the patients such that these patients need additional effective therapy, *e.g.*, remain unsusceptible to therapy. The phrase can also describe patients who respond to therapy yet suffer from side effects, relapse, develop resistance, *etc.* In various embodiments, "non-responsive/refractory" means that at least some significant portion of the cancer cells are not killed or their cell division arrested.

The determination of whether the cancer cells are "non-responsive/refractory" can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a cancer is "non-responsive/refractory" where the number of cancer cells has not been significantly reduced, or has increased during the treatment.

[0086] As used herein, the term "overexpress" in the context of EphA2 overexpression means that the gene encoding EphA2 is expressed at a level above that which is expressed by a normal human cell as assessed by an assay described herein or known to one of skill in the art (*e.g.*, an immunoassay such as an ELISA or Western blot, a Northern blot, or RT-PCR).

[0087] As used herein, the term "potentiate" refers to an improvement in the efficacy of a therapy at its common or approved dose.

[0088] As used herein, the terms "prevent," " preventing" and "prevention" refer to the prevention of the onset, recurrence, or spread of a disease in a subject resulting from the administration of a therapy (*e.g.*, prophylactic or therapeutic agent), or a combination of therapies.

[0089] As used herein, the term "prophylactic agent" refers to any agent that can be used in the prevention of the onset, recurrence or spread of a disorder associated with EphA2 overexpression, a disorder associated with aberrant angiogenesis and/or a hyperproliferative cell disease, particularly cancer. In certain embodiments, the term "prophylactic agent" refers to a *Listeria*-based EphA2 vaccine of the invention. In certain other embodiments, the term "prophylactic agent" refers to a therapy other than a *Listeria*-based EphA2 vaccine, *e.g.*, a cancer chemotherapeutic, radiation therapy, hormonal therapy, biological therapy (*e.g.*, immunotherapy). In other embodiments, more than one prophylactic agent may be administered in combination.

[0090] As used herein, a "prophylactically effective amount" refers to that amount of a therapy (*e.g.*, a prophylactic agent) sufficient to result in the prevention of the onset, recurrence or spread of a disorder (*e.g.*, a disorder associated with aberrant angiogenesis and a hyperproliferative cell disease, preferably, cancer). A prophylactically effective amount may refer to the amount of therapy (*e.g.*, a prophylactic agent) sufficient to prevent the onset, recurrence or spread of a disorder (*e.g.*, a disorder associated with aberrant angiogenesis and a hyperproliferative cell disease, particularly cancer) in a subject including, but not limited to, subjects predisposed to a hyperproliferative cell disease, for example, those genetically predisposed to cancer or previously exposed to carcinogens. A prophylactically effective amount may also refer to the amount of a therapy (*e.g.*,

prophylactic agent) that provides a prophylactic benefit in the prevention of a disorder (*e.g.*, a disorder associated with aberrant angiogenesis and a hyperproliferative cell disease). Further, a prophylactically effective amount with respect to a therapy (*e.g.*, prophylactic agent) means that amount of a therapy (*e.g.*, prophylactic agent) alone, or in combination with other therapies (*e.g.*, agents), that provides a prophylactic benefit in the prevention of a disorder (*e.g.*, a disorder associated with aberrant angiogenesis and a hyperproliferative cell disease). Used in connection with an amount of a *Listeria*-based EphA2 vaccine of the invention, the term can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of or synergies with another therapy (*e.g.*, a prophylactic agent).

[0091] A used herein, a "protocol" includes dosing schedules and dosing regimens.

[0092] As used herein, the phrase "side effects" encompasses unwanted and adverse effects of a prophylactic or therapeutic agent. Adverse effects are always unwanted, but unwanted effects are not necessarily adverse. An adverse effect from a therapy (*e.g.*, a prophylactic or therapeutic agent) might be harmful or uncomfortable or risky. Side effects from chemotherapy include, but are not limited to, gastrointestinal toxicity such as, but not limited to, early and late-forming diarrhea and flatulence, nausea, vomiting, anorexia, leukopenia, anemia, neutropenia, asthenia, abdominal cramping, fever, pain, loss of body weight, dehydration, alopecia, dyspnea, insomnia, dizziness, mucositis, xerostomia, and kidney failure, as well as constipation, nerve and muscle effects, temporary or permanent damage to kidneys and bladder, flu-like symptoms, fluid retention, and temporary or permanent infertility. Side effects from radiation therapy include but are not limited to fatigue, dry mouth, and loss of appetite. Side effects from biological therapies/immunotherapies include but are not limited to rashes or swellings at the site of administration, flu-like symptoms such as fever, chills and fatigue, digestive tract problems and allergic reactions. Side effects from hormonal therapies include but are not limited to nausea, fertility problems, depression, loss of appetite, eye problems, headache, and weight fluctuation. Additional undesired effects typically experienced by patients are numerous and known in the art. Many are described in the *Physicians' Desk Reference* (56th ed., 2002, 57th ed., 2003 and 58th ed., 2004).

[0093] As used herein, the terms "subject" and "patient" are used interchangeably. As used herein, a subject is preferably a mammal such as a non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats *etc.*) and a primate (*e.g.*, monkey and human), most preferably a human. In a specific embodiment, the subject is a non-human animal. In another embodiment, the subject is a farm animal (*e.g.*, a horse, a pig, a lamb or a cow) or a pet

(*e.g.*, a dog, a cat, a rabbit or a bird). In another embodiment, the subject is an animal other than a laboratory animal or animal model (*e.g.*, a mouse, a rat, a guinea pig or a monkey). In a preferred embodiment, the subject is a human. In another preferred embodiment, the subject is a human that is not immunocompromised or immunosuppressed. In another preferred embodiment, the subject is a human with a mean absolute lymphocyte count of approximately 500 cells/mm^3, approximately 600 cells/mm^3, approximately 650 cells/mm^3, approximately 700 cells/mm^3, approximately 750 cells/mm^3, approximately 800 cells/mm^3, approximately 850 cells/mm^3, approximately 900 cells/mm^3, approximately 950 cells/mm^3, approximately 1000 cells/mm^3, approximately 1050 cells/mm^3, approximately 1100 cells/mm^3, or approximately 1150 cells/mm^3 or approximately 1200 cells/mm^3.

[0094] As used herein, the terms "treat," "treating" and "treatment" refer to the eradication, reduction or amelioration of a disorder or a symptom thereof, particularly, the eradication, removal, modification, or control of primary, regional, or metastatic cancer tissue that results from the administration of one or more therapies (*e.g.*, therapeutic agents). In certain embodiments, such terms refer to the minimizing or delaying the spread of cancer resulting from the administration of one or more therapies (*e.g.*, therapeutic agents) to a subject with such a disease.

[0095] As used herein, the term "therapeutic agent" refers to any agent that can be used in the prevention, treatment, or management of a disease (*e.g.*, a disorder associated with overexpression of EphA2 and/or hyperproliferative cell disorder, particularly, cancer). In certain embodiments, the term "therapeutic agent" refers to a *Listeria*-based EphA2 vaccine of the invention. In certain other embodiments, the term "therapeutic agent" refers to a therapy other than a *Listeria*-based EphA2 vaccine such as, *e.g.*, a cancer chemotherapeutic, radiation therapy, hormonal therapy, and/or biological therapy/immunotherapy. In other embodiments, more than one therapy (*e.g.*, a therapeutic agent) may be administered in combination.

[0096] As used herein, a "therapeutically effective amount" refers to that amount of a therapy (*e.g.*, a therapeutic agent) sufficient to treat or manage a disorder (*e.g.*, a disorder associated with EphA2 overexpression, a disorder associated with aberrant angiogenesis and/or hyperproliferative cell disease) and, preferably, the amount sufficient to destroy, modify, control or remove primary, regional or metastatic cancer tissue. A therapeutically effective amount may refer to the amount of a therapy (*e.g.*, a therapeutic agent) sufficient to delay or minimize the onset of a disorder (*e.g.*, hyperproliferative cell disease), *e.g.*, delay or minimize the spread of cancer. A therapeutically effective amount may also refer to the amount of a therapy (*e.g.*, a therapeutic agent) that provides a therapeutic benefit in the

treatment or management of a disorder (*e.g.*, cancer). Further, a therapeutically effective amount with respect to a therapy (*e.g.*, a therapeutic agent) means that amount of a therapy (*e.g.*, therapeutic agent) alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or management of a disorder (*e.g.*, a hyperproliferative cell disease such as cancer). Used in connection with an amount of a *Listeria*-based EphA2 vaccine, the term can encompass an amount that improves overall therapy, reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergies with another therapy (*e.g.*, a therapeutic agent).

[0097] As used herein, the term "therapy" refers to any protocol, method and/or agent that can be used in the prevention, treatment or management of a disorder (*e.g.*, a hyperproliferative cell disorder, a disorder associated with aberrant angiogenesis and/or a non-neoplastic hyperproliferative cell disorder) or a symptom thereof. In certain embodiments, the terms "therapies" and "therapy" refer to a biological therapy, supportive therapy, and/or other therapies useful in treatment, management, prevention, or amelioration of a disorder (*e.g.*, a hyperproliferative cell disorder and/or a non-neoplastic hyperproliferative cell disorder) or one or more symptoms thereof known to one of skill in the art such as medical personnel.

[0098] As used herein, the term "synergistic" refers to a combination of therapies (*e.g.*, prophylactic or therapeutic agents) which is more effective than the additive effects of any two or more single therapies (*e.g.*, one or more prophylactic or therapeutic agents). A synergistic effect of a combination of therapies (*e.g.*, a combination of prophylactic or therapeutic agents) permits the use of lower dosages of one or more of therapies (*e.g.*, one or more prophylactic or therapeutic agents) and/or less frequent administration of said therapies to a subject with a non-neoplastic hyperproliferative epithelial and/or endothelial cell disorder. The ability to utilize lower dosages of therapies (*e.g.*, prophylactic or therapeutic agents) and/or to administer said therapies less frequently reduces the toxicity associated with the administration of said therapies to a subject without reducing the efficacy of said therapies in the prevention or treatment of a disorder (*e.g.*, a hyperproliferative cell disorder). In addition, a synergistic effect can result in improved efficacy of therapies (*e.g.*, prophylactic or therapeutic agents) in the prevention or treatment of a disorder (*e.g.*, a disorder associated with aberrant angiogenesis and a hyperproliferative cell disorder). Finally, synergistic effect of a combination of therapies (*e.g.*, prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of any single therapy.

[0099] As used herein, the terms "T cell malignancies" and "T cell malignancy" refer to any T cell lymphoproliferative disorder, including thymic and post-thymic malignancies. T cell malignancies include tumors of T cell origin. T cell malignancies refer to tumors of lymphoid progenitor cell, thymocyte, T cell, NK-cell, or antigen presenting cell origin. T cell malignancies include, but are not limited to, leukemias, including acute lymphoblastic leukemias, thymomas, acute lymphoblastic leukemias, and lymphomas, including Hodgkin's and non-Hodgkin's disease, with the proviso that T cell malignancies are not cutaneous T cell malignancies, in particular cutaneous-cell lymphomas. In a preferred embodiment, T cell malignancies are systemic, non-cutaneous T cell malignancies.

3.2. SEQUENCES

[00100] Below is a brief summary of the sequences presented in the accompanying sequence listing, which is incorporated by reference herein in its entirety:

[00101] **SEQ ID NO:1**

Human EphA2 cDNA (full length)

[00102] **SEQ ID NO:2**

Human EphA2 polypeptide (full length)

[00103] **SEQ ID NOs:3-18**

Human EphA2 peptides

[00104] **SEQ ID NO:19**

Construct: LLOss-PEST-hEphA2

Native LLO signal peptide + PEST fused to full-length human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Fusion protein coding sequence shown

[00105] **SEQ ID NO:20**

Construct: LLOss-PEST-hEphA2

Native LLO signal peptide + PEST fused to full-length human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[00106] **SEQ ID NO:21**

EphA2 EX2 domain

Native nucleotide sequence

[00107] **SEQ ID NO:22**

EphA2 EX2 domain

Nucleotide sequence for optimal codon usage in Listeria

[00108] **SEQ ID NO:23**

EphA2 EX2 domain

Primary Amino Acid Sequence

[00109] **SEQ ID NO:24**

Construct: LLOss-PEST-EX2_hEphA2

Native LLO signal peptide + PEST fused to external domain of human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

[00110] **SEQ ID NO:25**

Construct: LLOss-PEST-EX2_hEphA2

Native LLO signal peptide + PEST fused to external domain of human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[00111] **SEQ ID NO:26**

NativeLLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized - FLAG-EX-2 EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00112] **SEQ ID NO:27**

NativeLLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized - FLAG-EX-2 EphA2-Myc)

Primary Amino Acid Sequence

[00113] **SEQ ID NO:28**

Codon Optimized LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-EX-2 EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00114] **SEQ ID NO:29**

Codon Optimized LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-EX-2 EphA2-Myc)

Primary Amino Acid Sequence

[00115] **SEQ ID NO:30**

PhoD-FLAG-EX2_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-EX-2 EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00116] **SEQ ID NO:31**

PhoD-FLAG-EX2_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-EX-2 EphA2-Myc)

Amino acid sequence

[00117] **SEQ ID NO:32**

EphA2 CO domain

Native nucleotide sequence

[00118] **SEQ ID NO:33**

EphA2 CO domain

Nucleotide sequence for optimal codon usage in Listeria

[00119] **SEQ ID NO:34**

EphA2 CO domain

Primary Amino Acid Sequence

[00120] **SEQ ID NO:35**

Construct: LLOss-PEST-CO-huEphA2

Native LLO signal peptide + PEST fused to cytoplasmic domain of human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Fusion protein coding sequence shown

[00121] **SEQ ID NO:36**

Construct: LLOss-PEST-CO-huEphA2

Native LLO signal peptide + PEST fused to cytoplasmic domain of human EphA2

Not Codon optimized

No epitope tags (e.g., myc or FLAG used in this construct)

Predicted fusion protein shown

[00122] **SEQ ID NO:37**

NativeLLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-CO_EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00123] **SEQ ID NO:38**

NativeLLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Native L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-CO_EphA2-Myc)

Primary Amino Acid Sequence

[00124] **SEQ ID NO:39**

Codon Optimized LLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-CO_EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00125] **SEQ ID NO:40**

Codon Optimized LLOss-PEST-FLAG-CO_EphA2-myc-CodonOp

(Codon Optimized L. monocytogenes LLO signal peptide + PEST-Codon optimized -FLAG-CO_EphA2-Myc)

Primary Amino Acid Sequence

[00126] **SEQ ID NO:41**

PhoD-FLAG-CO_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-CO_EphA2-Myc)

Nucleotide Sequence (including *hly* promoter)

[00127] **SEQ ID NO:42**

PhoD-FLAG-CO_EphA2-myc-CodonOp

(Codon optimized B. subtilis phoD Tat signal peptide-FLAG-CO_EphA2-Myc)

Amino acid sequence

[00128] **SEQ ID NO:43**

Construct: pAM401-MCS

Plasmid pAM401 containing multiple cloning site (MCS) from pPL2 vector

Insertion of small *Aat II* MCS fragment from pPL2 inserted into pAM401 plasmid between blunted *Xba I* and *Nru I* sites.

Complete pAM401-MCS plasmid sequence shown

4. BRIEF DESCRIPTION OF THE FIGURES

[00129] **Figure 1.** *Listeria* intracellular life cycle, antigen presenting cell activation, and antigen presentation.

[00130] **Figure 2.** Western blot analysis of secreted protein from recombinant *Listeria* encoding native EphA2 CO domain sequence.

[00131] **Figure 3.** Western blot analysis of secreted protein from recombinant *Listeria* encoding native or codon-optimized LLO secA1 signal peptide fused with codon-optimized EphA2 EX2 domain sequence signal peptide.

[00132] **Figure 4.** Western blot analysis of secreted protein from recombinant *Listeria* encoding native or codon-optimized LLO secA2 signal peptide or codon-optimized Tat signal peptide fused with codon-optimized EphA2 CO domain sequence.

[00133] **Figure 5.** Flow cytometry analysis of human EphA2 expression in CT2 murine carcinoma cells. Single cell FACS sorting assays were performed by standard techniques to identify CT26 cell clones expressing high levels of human EphA2.

[00134] **Figure 6.** Western blot analysis of pooled populations CT26 murine colon carcinoma cells expressing high levels of human EphA2 protein.

[00135] **Figure 7.** Flow Cytometry of B16F10 cells expressing huEphA2.

[00136] **Figure 8.** Western blot analysis of lysate from 293 cells 48 hr. following transfection with pCDNA4 plasmid DNA encoding full-length native EphA2 sequence.

[00137] **Figures 9A-9B.** In the CT26 tumor model, therapeutic immunization with positive control *Listeria* expressing AH1-A5.

[00138] **Figures 10A-10B.** Preventative immunization with *Listeria* expressing ECD of hEphA2 suppresses CT26-hEphA2 tumor growth (Figure 10A) and increases survival (Figure 10B).

[00139] **Figures 11A-11D.** Preventive studies following i.v. administration of L4029EphA2-exFlag, *Listeria* control (L4029), or *Listeria* positive control containing the AH1 protein (L4029-AH1) ($5x10^5$ cells in 100 μl volume) either subcutaneously or intravenously. **Figure 11A** demonstrates tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2, vehicle (HBSS), *Listeria* (L4029) or *Listeria* positive (L4029-AH1) controls. **Figure 11B** demonstrates mean tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 (L4029-EphA2 exFlag) compared to the

Listeria (L4029) control. **Figure 11C** illustrates results of the prevention study in the s.c. model, measuring percent survival of the mice post CT26 tumor cell inoculation. **Figure 11D** illustrates the results of the prevention study in the lung metastases model, measuring percent survival of the mice post tumor cell inoculation.

[00140] **Figure 12.** Preventative immunization with Listeria expressing ECD of hEphA2 increases survival following RenCa-hEphA2 tumor challenge.

[00141] **Figures 13A-13C. Figures 13A-13C** illustrate results of a typical therapeutic study of animals inoculated with CT26 murine colon carcinoma cells transfected with human EphA2 (L4029-EphA2 exFlag), Listeria control (L4029-control) or vehicle (HBSS). In **Figure 13A,** tumor volume was measured at several intervals post inoculation. **Figure 13B** illustrates the mean tumor volume of mice inoculated with CT26 cells containing either Listeria control or the ECD of huEphA2. **Figure 13C** represents the results of a therapeutic study using the lung metastases model, measuring percent survival of mice post inoculation with CT26 cells with either HBSS or Listeria control, or Listeria expressing the ECD of huEphA2.

[0001] **Figures 14A-F. Figure 14A.** Therapeutic immunization in Balb/C mice with Listeria expressing ICD of hEphA2 suppresses established CT26-hEphA2 tumor growth. **Figure 14B.** Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with recombinant Listeria encoding EphA2 CO domain confers long-term survival. **Figure 14C.** Long-term survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors immunized with recombinant Listeria encoding OVA.AH1 or OVA.AH1-A5. **Figure 14D.** Increased survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors when immunized with recombinant Listeria encoding codon-optimized or native EphA2 CO domain sequence. **Figure 14E.** Increased survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors when immunized with recombinant Listeria encoding codon-optimized secA1 signal peptide fused with codon-optimized EphA2 EX2 domain sequence. **Figure 14F.** Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with recombinant Listeria encoding EphA2 CO domain but not with plasmid DNA encoding full-length EphA2 confers long-term survival.

[0002] **Figure 15.** Long-term suppression of CT26-hEphA2 tumor growth upon rechallenge.

[0003] **Figure 16.** Immunization with Listeria expressing hEphA2 elicits a specific CD8+ T cell response.

[0004] **Figure 17.** Both CD4+ and CD8+ T cell responses are required for optimal hEphA2-directed anti-tumor efficacy.

[00142] **Figures 18A-B.** Therapeutic vaccination with Listeria expressing human EphA2 ICD enhances CD45+ tumor infiltrate. **Figure 18A** depicts images of tumor sections stained with biotinylated rat anti-mouse CD45/B200. **Figure 18B** is a bar graph normalizing the image data to tumor volume.

5. DETAILED DESCRIPTION OF THE INVENTION

[00143] The present invention is based, in part, on the inventors' discovery that a *Listeria*-based vaccine comprising *Listeria* engineered to express EphA2 antigenic peptides can confer beneficial therapeutic and prophylactic benefits against hyperproliferative diseases involving EphA2-expressing cells.

[00144] The present invention provides methods and compositions that provide for the prevention, treatment, inhibition, and management of disorders associated with overexpression of EphA2, disorders associated with aberrant angiogenesis and/or hyperproliferative cell disorders. A particular aspect of the invention relates to methods and compositions containing compounds that, when administered to a subject with a hyperproliferative cell disorder involving EphA2-expressing cells, either elicit or mediate an immune response against EphA2, resulting in a growth inhibition of the EphA2-expressing cells involved in the hyperproliferative cell disorder. The present invention further relates to methods and compositions for the treatment, inhibition, or management of metastases of cancers of epithelial cell origin, especially human cancers of the breast, ovarian, esophageal, lung, skin, prostate, bladder, and pancreas, and renal cell carcinomas and melanomas. The invention further relates to methods and compositions for the prevention, treatment, inhibition, or management of cancers of T cell origin, especially leukemias and lymphomas. Further, the compositions and methods of the invention include other types of active ingredients in combination with the *Listeria*-based EphA2 vaccines of the invention. In certain embodiments, the compositions of the invention are used to treat, prevent or manage other non-neoplastic hyperproliferative cell disorders, for example, but not limited to asthma, psoriasis, restenosis, COPD, *etc.*

[00145] The present invention also relates to methods for the treatment, inhibition, and management of cancer and other hyperproliferative cell disorders that have become partially or completely refractory to current or standard therapy (*e.g.*, a cancer therapy, such as chemotherapy, radiation therapy, hormonal therapy, and biological/immunotherapy).

5.1. *Listeria*-Based Vaccines

[00146] The present invention provides *Listeria* bacteria engineered to express an EphA2 antigenic peptide and the use of such *Listeria* to manage, treat or prevent diseases associated with overexpression of EphA2 and/or hyperproliferative cell disorders.

[00147] A *Listeria*-based EphA2 vaccine may comprise one or more strains of *Listeria* that express an EphA2 antigenic peptide. In other embodiments, a *Listeria*-based EphA2 vaccine may comprise a *Listeria* strain that has been engineered to express one or more EphA2 antigenic peptides.

[00148] In a preferred embodiment, the *Listeria*-based EphA2 vaccine of the invention comprises the species *Listeria monocytogenes*.

5.1.1. Attenuation

[00149] To allow the safe use of *Listeria* in treatment of humans and animals, the bacteria are preferably attenuated in their virulence for causing disease. The end result is to reduce the risk of toxic shock or other side effects due to administration of the *Listeria* to the patient. Such attenuated *Listeria* can be isolated by a number of techniques. Such methods include use of antibiotic-sensitive strains of microorganisms, mutagenesis of the microorganisms, selection for microorganism mutants that lack virulence factors, and construction of new strains of microorganisms with altered cell wall lipopolysaccharides.

[00150] In certain embodiments, the *Listeria* can be attenuated by the deletion or disruption of DNA sequences which encode for virulence factors which insure survival of the *Listeria* in the host cell, especially macrophages and neutrophils, by, for example, homologous recombination techniques and chemical or transposon mutagenesis. Many, but not all, of these studied virulence factors are associated with survival in macrophages such that these factors are specifically expressed within macrophages due to stress, for example, acidification, or are used to induced specific host cell responses, for example, macropinocytosis, Fields *et al.*, 1986, *Proc. Natl. Acad. Sci.* USA 83:5189-5193. Examples of virulence genes include, but are not limited to, *hly*, *plcA*, *plcB*, *mpl*, *actA*, *inlA*, and *inlB*. *See also* Autret et al., 2001, Infection and Immunity 69:2054-2065.

[00151] In a specific embodiment, the *Listeria* are attenuated in their tissue tropism (*e.g.*, inlB mutant) and/or in their ability to spread from cell to cell (*e.g.*, actA mutant). In another embodiment, the *Listeria* comprise a mutation (*e.g.*, a deletion, addition or substitution) in one or more internalins (*e.g.*, inlA and/or inlB). In another embodiment, the *Listeria* comprise a mutation (*e.g.*, a deletion, addition or substitution) in actA.

[00152] As a method of insuring the attenuated phenotype and to avoid reversion to the non-attenuated phenotype, the *Listeria* may be engineered such that it is attenuated in

more than one manner, *e.g.*, a mutation affecting tissue tropism (*e.g.*, inlB mutant) and a mutation affecting the ability to spread from cell to cell (*e.g.*, actA mutant). In a preferred embodiment, the *Listeria* comprise a mutation (*e.g.*, a deletion, addition or substitution) in internalin B and a mutation in actA.

5.1.2. Expression Systems

[00153] The EphA2 antigenic peptides are preferably expressed in *Listeria* using a heterologous gene expression cassette. A heterologous gene expression cassette is typically comprised of the following ordered elements: (1) prokaryotic promoter; (2) Shine-Dalgarno sequence; (3) secretion signal (signal peptide); and, (4) heterologous gene. Optionally, the heterologous gene expression cassette may also contain a transcription termination sequence, in constructs for stable integration within the bacterial chromosome. While not required, inclusion of a transcription termination sequence as the final ordered element in a heterologous gene expression cassette may prevent polar effects on the regulation of expression of adjacent genes, due to read-through transcription.

[00154] The expression vectors introduced into the *Listeria*-based EphA2 vaccine are preferably designed such that the *Listeria*-produced EphA2 peptides and, optionally, a second tumor antigen, are secreted by the *Listeria*. A number of bacterial secretion signals are well known in the art and may be used in the compositions and methods of the present invention. An exemplary secretion signals that can be used with *Listeria* is SecA, as described in Section 5.2.1.4, *infra*.

[00155] The promoters driving the expression of the EphA2 antigenic peptides may be either constitutive, in which the peptides are continually expressed; inducible, in which the peptides are expressed only upon the presence of an inducer molecule(s); or cell-type specific, in which the peptides or enzymes are expressed only in certain cell types.

[00156] Preferred embodiments of components of the EphA2 antigenic peptide expression system, to be used in conjunction with nucleic acids encoding EphA2 antigenic peptides described in Section 5.2, are provided below.

5.1.2.1. Construct Backbone

[00157] One of ordinary skill in the art will recognize that a variety of plasmid construct backbones are available which are suitable for use in the assembly of a heterologous gene expression cassette. A particular plasmid construct backbone is selected based on whether expression of the heterologous gene expression cassette from the bacterial chromosome or from an extra-chromosomal episome is desired.

[00158] Given as non-limiting examples, incorporation of the heterologous gene expression cassette into the bacterial chromosome of *Listeria monocytogenes* (*Listeria*) is accomplished with an integration vector that contains an expression cassette for a listeriophage integrase that catalyzes sequence-specific integration of the vector into the *Listeria* chromosome. For example, the integration vectors known as pPL1 and pPL2 program stable single-copy integration of a heterologous protein (e.g., EphA2-antigenic peptide) expression cassette within an innocuous region of the bacterial genome, and have been described in the literature (Lauer et al., 2002, J. Bacteriol. 184:4177-4178). The integration vectors are stable as plasmids in *E. coli* and are introduced via conjugation into the desired *Listeria* background. Each vector lacks a *Listeria*-specific origin of replication and encodes a phage integrase, such that the vectors are stable only upon integration into a chromosomal phage attachment site. Starting with a desired plasmid construct, the process of generating a recombinant *Listeria* strain expressing a desired protein(s) takes approximately one week. The pPL1 and pPL2 integration vectors are based, respectively, on the U153 and PSA listeriophages. The pPL1 vector integrates within the open reading frame of the comK gene, while pPL2 integrates within the tRNAArg gene in such a manner that the native sequence of the gene is restored upon successful integration, thus keeping its native expressed function intact. The pPL1 and pPL2 integration vectors contain a multiple cloning site sequence in order to facilitate construction of plasmids containing the heterologous protein (e.g., EphA2-antigenic peptide) expression cassette.

[00159] Alternatively, incorporation of the EphA2-antigenic peptide expression cassette into the *Listeria* chromosome can be accomplished through alleleic exchange methods, known to those skilled in the art. In particular, compositions in which it is desired to not incorporate a gene encoding an antibiotic resistance protein as part of the construct containing the heterologous gene expression cassette, methods of allelic exchange are desirable. For example, the pKSV7 vector (Camilli *et al.*, 1993, *Mol. Microbiol.* 8:143-157), contains a temperature-sensitive *Listeria* Gram-positive replication origin which is exploited to select for recombinant clones at the non-permissive temperature that represent the pKSV7 plasmid recombined into the *Listeria* chromosome. The pKSV7 allelic exchange plasmid vector contains a multiple cloning site sequence in order to facilitate construction of plasmids containing the heterologous protein (*e.g.*, EphA2-antigenic peptide) expression cassette, and also a chloramphenicol resistance gene. For insertion into the *Listeria* chromosome, the heterologous EphA2-antigenic peptide expression cassette construct is optimally flanked by approximately 1 kb of chromosomal DNA sequence that corresponds to the precise location of desired integration. The pKSV7-heterologous protein

(*e.g.*, EphA2-antigenic peptide) expression cassette plasmid is introduced optimally into a desired bacterial strain by electroporation, according to standard methods for electroporation of Gram positive bacteria. Briefly, bacteria electroporated with the pKSV7-heterologous protein (*e.g.*, EphA2-antigenic peptide) expression cassette plasmid are selected by plating on BHI agar media containing chloramphenicol (10 μg/ml), and incubated at the permissive temperature of 30°C. Single cross-over integration into the bacterial chromosome is selected by passaging several individual colonies for multiple generations at the non-permissive temperature of 41°C in media containing chloramphenicol. Finally, plasmid excision and curing (double cross-over) is achieved by passaging several individual colonies for multiple generations at the permissive temperature of 30°C in BHI media not containing chloramphenicol. Verification of integration of the heterologous protein (*e.g.*, EphA2-antigenic peptide) expression cassette into the bacteria chromosome can be accomplished by PCR, utilizing a primer pair that amplifies a region defined from within the heterologous protein (*e.g.*, EphA2-antigenic peptide) expression cassette to the bacterial chromosome targeting sequence not contained in the pKSV7 plasmid vector construct.

[00160] In other compositions, it may be desired to express the heterologous protein (*e.g.*, EphA2-antigenic peptide) from a stable plasmid episome. Maintenance of the plasmid episome through passaging for multiple generations requires the co-expression of a protein that confers a selective advantage for the plasmid-containing bacterium. As non-limiting examples, the protein co-expressed from the plasmid in combination with the heterologous protein (*e.g.*, EphA2-antigenic peptide) may be an antibiotic resistance protein, for example chloramphenicol, or may be a bacterial protein (that is expressed from the chromosome in wild-type bacteria), that can also confer a selective advantage. Non-limiting examples of bacterial proteins include enzyme required for purine or amino acid biosynthesis (selection under defined media lacking relevant amino acids or other necessary precursor macromolecules), or a transcription factor required for the expression of genes that confer a selective advantage in vitro or in vivo (Gunn *et al.*, 2001, *J. Immuol.* 167:6471-6479). As a non-limiting example, pAM401 is a suitable plasmid for episomal expression of a selected heterologous protein (*e.g.*, EphA2-antigenic peptide) in diverse Gram-positive bacterial genera (Wirth *et al.*, 1986, *J. Bacteriol* 165:831-836).

5.1.2.2. <u>Shine-Dalgarno Sequence</u>

[00161] At the 3' end of the promoter is contained a poly-purine Shine-Dalgarno sequence, the element required for engagement of the 30S ribosomal subunit (via 16S rRNA) to the heterologous gene RNA transcript and initiation of translation. The Shine-

Dalgarno sequence has typically the following consensus sequence (SEQ ID NO:66): 5'-NAGGAGGU-N5-10-AUG (start codon)-3'. There are variations of the poly-purine Shine-Dalgarno sequence Notably, the *Listeria* hly gene that encodes listerolysin O (LLO) has the following Shine-Dalgarno sequence (SEQ ID NO:67): A<u>AGGAGA</u>GTGAAACCC**ATG** (Shine-Dalgarno sequence is underlined, and the translation start codon is bolded).

5.1.2.3. Codon Optimization

[00162] In some embodiments, for optimal translation efficiency of a selected heterologous protein, it is desirable to utilize codons favored by Listeria. The preferred codon usage for bacterial expression can be determined as described in Nakamura et al., 2000, *Nucl. Acids Res.* 28:292. In some embodiments, codon-optimized expression of EphA2 antigenic peptides, from *Listeria monocytogenes* is desired.

[00163] The optimal codons utilized by *Listeria monocytogenes* for each amino acid are shown in Table 3 below.

Amino Acid	One Letter Code	Optimal *Listeria* Codon
Alanine	A	GCA
Arginine	R	CGU
Asparagine	N	AAU
Aspartate	D	GAU
Cysteine	C	UGU
Glutamine	Q	CAA
Glutamate	E	GAA
Glycine	G	GGU
Histidine	H	CAU
Isoleucine	I	AUU
Leucine	L	UUA
Lysine	K	AAA
Methionine	M	AUG
Phenylalanine	F	UUU
Proline	P	CCA
Serine	S	AGU
Threonine	T	ACA
Tryptophan	W	UGG
Tyrosine	Y	UAU
Valine	V	GUU

[00164] **TABLE 3**: *Listeria* Codon Bias: Codons to be used for optimizing expression

5.1.2.4. Signal Peptides

[00165] Bacteria utilize diverse pathways for protein secretion, including secA1 and Twin-Arg Translocation (Tat), which are located at the N-terminal end of the pre-protein. The majority of secreted proteins utilize the Sec pathway, in which the protein translocates through the bacterial membrane-embedded proteinaceous Sec pore in an unfolded conformation. In contrast, the proteins utilizing the Tat pathway are secreted in a folded conformation.

[00166] Nucleotide sequence encoding signal peptides corresponding to either of these protein secretion pathways (including, but not limited to, the signal peptides described in Section 5.1.2.4 and the signal and leader peptides described in Section 5.2.1) can be fused genetically in-frame to a desired heterologous protein coding sequence. The signal peptides optimally contain a signal peptidase at their carboxyl terminus for release of the authentic desired protein into the extra-cellular environment (Sharkov and Cai, 2002, J. Biol. Chem. 277:5796-5803; Nielsen et al., 1997, Protein Engineering 10:1-6). Signal peptide cleavage sites can be predicted using programs such as SignalP 3.0 (Bendtsen *et al.*, 2004, J. Mol. Biol. 340:783-795. The signal peptides can be derived not only from diverse secretion pathways, but also from diverse bacterial genera. Signal peptides have a common structural organization, having a charged N-terminus (N-domain), a hydrophobic core region (H-domain) and a more polar C-terminal region (C-domain), however, they do not show sequence conservation. The C-domain of the signal peptide carries a type I signal peptidase (SPase I) cleavage site, having the consensus sequence A-X-A, at positions –1 and –3 relative to the cleavage site. Proteins secreted via the sec pathway have signal peptides that average 28 residues. Signal peptides related to proteins secreted by the Tat pathway have a tripartite organization similar to Sec signal peptides, but are characterized by having an RR-motif (R-R-X-#-#, where # is a hydrophobic residue), located at the N-domain / H-domain boundary. Bacterial Tat signal peptides average 14 amino acids longer than sec signal peptides. The Bacillus subtilis secretome may contain as many as 69 putative proteins that utilize the Tat secretion pathway, 14 of which contain a SPase I cleavage site (Jongbloed *et al.*, 2002, J. Biol. Chem. 277:44068-44078; Thalsma *et al.*, 2000, Microbiol. Mol. Biol. Rev. 64:515-547). Shown in Table 4 below are non-limiting examples of signal peptides that can be used in fusion compositions with a selected heterologous gene, resulting in secretion from the bacterium of the encoded protein.

Secretion Pathway	Signal Peptide Amino Acid Sequence (NH_2-CO_2)	Signal peptidase Site (cleavage site represented by ')	Gene	Genus/species	SEQ ID NO:
secA1	MKKIMLVFITLILVSLPI AQQTEAKD	TEA'KD (SEQ ID NO:70)	*hly* (LLO)	*Listeria monocytogene*	44

				s	
Tat	MTDKKSENQTEKTETKENKGMTRREMLKLSAVAGTGIAVGATGLGTILNVVDQVDKALT	DKA'LT (SEQ ID NO:71)	lmo0367	*Listeria monocytogenes*	45
	MAYDSRFDEWVQKLKEESFQNNTFDRRKFIQGAGKIAGLSLGLTIAQSVGAFG	VGA'FG (SEQ ID NO:72)	PhoD (alkaline phosphatase)	*Bacillus subtillis*	46

[00167] **TABLE 4**: signal sequences useful for bacterial expression and secretion of EphA2.

[00168] There are a variety of proteins among diverse bacterial genera that are secreted via the Tat pathway . In some embodiments, selected Tat signal peptides corresponding to these proteins are fused genetically in-frame to a desired sequence encoding an EphA2 antigenic peptide, to facilitate secretion of the functionally linked Tat signal peptide-EphA2 protein chimera via the Tat pathway. Provided below are non-limiting examples of proteins from *Bacillus subtilis* and *Listeria* (*innocua* and *monocytogenes*) that are predicted to utilize Tat pathway secretion.

<u>Putative *Bacillus subtilis* Proteins Secreted by Tat</u>

[00169] >gi|2635523|emb|CAB15017.1| similar to two (component sensor histidine kinase (YtsA) (Bacillus subtilis)

[00170] >gi|2632548|emb|CAB12056.1| phosphodiesterase/alkaline phosphatase D (Bacillus subtilis)

[00171] >gi|2632573|emb|CAB12081.1| similar to hypothetical proteins (Bacillus subtilis)

[00172] >gi|2633776|emb|CAB13278.1| similar to hypothetical proteins (Bacillus subtilis)

[00173] >gi|2634674|emb|CAB14172.1| menaquinol:cytochrome c oxidoreductase (iron (sulfur subunit) (Bacillus subtilis)

[00174] >gi|2635595|emb|CAB15089.1| yubF (Bacillus subtilis)

[00175] >gi|2636361|emb|CAB15852.1| alternate gene name: ipa (29d~similar to hypothetical proteins (Bacillus subtilis)

[00176] Putative *Listeria* Proteins Secreted by Tat

[00177] >gi|16799463|ref|NP_469731.1| conserved hypothetical protein similar to B. subtilis YwbN protein (*Listeria* innocua)

[00178] >gi|16801368|ref|NP_471636.1| similar to 3 (oxoacyl (acyl (carrier protein synthase (*Listeria* innocua)

[00179] <u>*Listeria monocytogenes EGD (e)*</u>

[00180] >gi|16802412|ref|NP_463897.1| conserved hypothetical protein similar to B. subtilis YwbN protein (*Listeria* monocytogenes EGD (e)

[00181] Organisms utilize codon bias to regulate expression of particular endogenous genes. Thus, signal peptides utilized for secretion of selected heterologous proteins may not contain codons that utilize preferred codons, resulting in non-optimal levels of protein synthesis. In some some embodiments, the signal peptide sequence fused in frame with a gene encoding a selected heterologous protein is codon-optimized for codon usage in a selected bacterium. In some embodiments for expression and secretion from recombinant *Listeria monocytogenes*, a nucleotide sequence of a selected signal peptide is codon optimized for expression in *Listeria monocytogenes*, according to Table 4, *supra*.

5.1.2.5. Transcription Termination Sequence

[00182] In some embodiments, a transcription termination sequence can be inserted into the heterologous protein expression cassette, downstream from the C-terminus of the translational stop codon related to the heterologous protein. Appropriate sequence elements known to those who are skilled in the art that promote either rho-dependent or rho-independent transcription termination can be placed in the heterologous protein expression cassette.

5.2. EphA2 Antigenic Peptides

[00183] As discussed above, the present invention relates to the use of *Listeria* that have been engineered to express an EphA2 antigenic peptide. Without being bound by any mechanism, such *Listeria* are capable of eliciting an immune response to EphA2 upon administration to a subject with a disease involving overexpression of EphA2, resulting in a cellular or humoral immune response against endogenous EphA2.

[00184] In principle, an EphA2 antigenic peptide (sometimes referred to as an "EphA2 antigenic polypeptide") for use in the methods and compositions of the present invention can be any EphA2 antigenic peptide that is capable of eliciting an immune response against EphA2-expressing cells involved in a hyperproliferative disorder. Thus, an EphA2 antigenic peptide can be an EphA2 polypeptide, preferably an EphA2 polypeptide of SEQ ID NO:2, or a fragment or derivative of an EphA2 polypeptide that (1) displays antigenicity of EphA2 (ability to bind or compete with EphA2 for binding to an anti-EphA2 antibody, (2) displays immunogenicity of EphA2 (ability to generate antibody which binds to EphA2), and/or (3) contains one or more T cell epitopes of EphA2.

[00185] In certain embodiments, the EphA2 antigenic peptide is a sequence encoded by the nucleotide sequence provided below or a fragment or derivative thereof:

[00186]	Genbank Accession No. NM_004431	Human
[00187]	Genbank Accession No. NM_010139	Mouse
[00188]	Genbank Accession No. AB038986	Chicken (partial)

[00189] In certain embodiments, the EphA2 antigenic peptide is full length human EphA2 (SEQ ID NO:2).

[00190] In other embodiments, the EphA2 antigenic peptide comprises the intracellular domain of EphA2 (residue 22 to 554 of SEQ ID NO:2).

[00191] In yet other embodiments, the EphA2 antigenic peptide comprises the instracellular domain EphA2 (residue 558 to 976 of SEQ ID NO:2).

[00192] In yet other embodiments, the EphA2 antigenic peptide comprises more than one domain of the full length human EphA2. In a specific embodiment, the EphA2 antigenic peptides comprises the extracellular domain and the intracellular cytoplasmic domain, joined together. In accordance with this embodiment, the transmembrane domain of EphA2 is deleted.

[00193] In certain embodiments of the invention, the tyrosine kinase activity of EphA2 is ablated. Thus, EphA2 may contain deletions, additions or substitutions of amino acid residues that result in the elimination of tyrosine kinase activity. In a preferred embodiment, a lysine to methione substitution at position 646 is present.

[00194] In a preferred embodiment, the EphA2 antigenic peptide comprises the extracellular and cytoplasmic domains of EphA2 resulting from a deletion of the transmembrane domain of EphA2 and has a lysine to methionine substitution as position 646.

[00195] In certain embodiments, the peptide corresponds to or comprises an EphA2 epitope that is exposed in a cancer cell but occluded in a non-cancer cell. In a preferred embodiment, the EphA2 antigenic peptides preferentially include epitopes on EphA2 that are selectively exposed or increased on cancer cells but not non-cancer cells ("exposed EphA2 epitope peptides").

[00196] The present invention further encompasses the use of a plurality of EphA2 antigenic peptides, *e.g.*, 2, 3, 4, 5, 6, or more EphA2 antigenic peptides, in the compositions and methods of the present invention.

[00197] Fragments of EphA2 that are useful in the methods and compositions present invention may contain deletions, additions or substitutions of amino acid residues within the amino acid sequence encoded by an EphA2 gene. Preferably mutations result in a silent change, thus producing a functionally equivalent EphA2 gene product. By "functionally

equivalent", it is meant that the mutated EphA2 gene product has the same function as the wild-type EphA2 gene product, e.g., contains one or more epitopes of EphA2.

[00198] An EphA2 antigenic peptide sequence preferably comprises an amino acid sequence that exhibits at least about 65% sequence similarity to human EphA2, more preferably exhibits at least 70% sequence similarity to human EphA2, yet more preferably exhibits at least about 75% sequence similarity human EphA2. In other embodiments, the EphA2 polypeptide sequence preferably comprises an amino acid sequence that exhibits at least 85% sequence similarity to human EphA2, yet more preferably exhibits at least 90% sequence similarity to human EphA2, and most preferably exhibits at least about 95% sequence similarity to human EphA2.

[00199] Additional polypeptides suitable in the present methods are those encoded by the nucleic acids described in Section 5.2 below.

[00200] The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, *Proc Natl Acad Sci. USA* 87:2264-2268, modified as in Karlin and Altschul, 1993, *Proc Natl Acad Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*,1990, *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

[00201] Another preferred, non limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *Comput Appl Biosci* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM

as described in Torellis and Robotti, 1994, *Comput. Appl. Biosci.* 10:3-5; and FASTA described in Pearson and Lipman,1988, Proc Natl Acad Sci USA 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search. If ktup = 2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup = 1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA. For a further description of FASTA parameters, *see* http://bioweb.pasteur.fr/docs/man/man/fasta.1.html#sect2.

[00202] The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted. However, conservative substitutions should be considered in evaluating sequences that have a low percent identity with the EphA2 sequences disclosed herein.

[00203] In a specific embodiment, EphA2 antigenic peptides comprising at least 10, 20, 30, 40, 50, 75, 100, or 200 amino acids of an EphA2 polypeptide, preferably of SEQ ID NO:2 are used in the present invention. In a preferred embodiment, EphA2 antigenic peptides comprising at least 10, 20, 30, 40, 50, 75, 100, or 200 continguous amino acids of an EphA2 polypeptide, preferably of SEQ ID NO:2 are used in the present invention. In a preferred embodiment, such a polypeptide comprises all or a portion of the extracellular domain of an EphA2 polypeptide of SEQ ID NO:2.

5.2.1. <u>FUSION PROTEINS</u>

[00204] In certain embodiments of the present invention, a *Listeria*-based EphA2 vaccine expresses an EphA2 antigenic peptide that is a fusion protein. Thus, the present invention encompasses compositions and methods in which the EphA2 antigenic peptides are fusion proteins comprising all or a fragment or derivative of EphA2 operatively associated to a heterologous component, *e.g.*, a heterologous peptide. Heterologous components can include, but are not limited to sequences which facilitate isolation and purification of the fusion protein. Heterologous components can also include sequences which confer stability to EphA2 antigenic peptides. Such fusion partners are well known to those of skill in the art.

[00205] The present invention encompasses the use of fusion proteins comprising an EphA2 polypeptide (*e.g.*, a polypeptide of SEQ ID NO:2 or a fragment thereof) and a heterologous polypeptide (*i.e.*, a polypeptide or fragment thereof, preferably a fragment of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 contiguous amino acids of the polypeptide). The fusion can be

direct, but may occur through linker sequences. The heterologous polypeptide may be fused to the N-terminus or C-terminus of the EphA2 antigenic peptide. Alternatively, the heterologous polypeptide may be flanked by EphA2 polypeptide sequences

[00206] A fusion protein can comprise an EphA2 antigenic peptide fused to a heterologous signal sequence at its N-terminus. Various signal sequences are commercially available. In addition to the signal sequences described in Section 5.1.2.4 *supra*, prokaryotic heterologous signal sequences useful in the methods of the invention include, but are not limited to, the phoA secretory signal (Sambrook *et al.*, eds., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) and the protein A secretory signal (Pharmacia Biotech, Piscataway, NJ).

[00207] The EphA2 antigenic peptide can be fused to tag sequences, *e.g.*, a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA), among others, many of which are commercially available for use in the methods of the invention. As described in Gentz *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other examples of peptide tags are the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, *Cell*, 37:767) and the "flag" tag (Knappik *et al.*, 1994, *Biotechniques*, 17(4):754-761). These tags are especially useful for purification of recombinantly produced EphA2 antigenic peptides.

[00208] Any fusion protein may be readily purified by utilizing an antibody specific or selective for the fusion protein being expressed. For example, a system described by Janknecht *et al.* allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88:8972). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

[00209] An affinity label can also be fused at its amino terminal to the carboxyl terminal of the EphA2 antigenic peptide for use in the methods of the invention. The precise site at which the fusion is made in the carboxyl terminal is not critical. The optimal site can be determined by routine experimentation. An affinity label can also be fused at its carboxyl terminal to the amino terminal of the EphA2 antigenic peptide for use in the methods and compositions of the invention.

[00210] A variety of affinity labels known in the art may be used, such as, but not limited to, the immunoglobulin constant regions (*see also* Petty, 1996, Metal-chelate affinity chromatography, in Current Protocols in Molecular Biology, Vol. 2, Ed. Ausubel *et al.*, Greene Publish. Assoc. & Wiley Interscience), glutathione S-transferase (GST; Smith, 1993, *Methods Mol. Cell Bio.* 4:220-229), the *E. coli* maltose binding protein (Guan *et al.*, 1987, *Gene* 67:21-30), and various cellulose binding domains (U.S. Patent Nos. 5,496,934; 5,202,247; 5,137,819; Tomme *et al.*, 1994, *Protein Eng.* 7:117-123), *etc.* Other affinity labels are recognized by specific binding partners and thus facilitate isolation by affinity binding to the binding partner which can be immobilized onto a solid support. Some affinity labels may afford the EphA2 antigenic peptide novel structural properties, such as the ability to form multimers. These affinity labels are usually derived from proteins that normally exist as homopolymers. Affinity labels such as the extracellular domains of CD8 (Shiue *et al.*, 1988, *J. Exp. Med.* 168:1993-2005), or CD28 (Lee *et al.*, 1990, *J. Immunol.* 145:344-352), or fragments of the immunoglobulin molecule containing sites for interchain disulfide bonds, could lead to the formation of multimers.

[00211] As will be appreciated by those skilled in the art, many methods can be used to obtain the coding region of the above-mentioned affinity labels, including but not limited to, DNA cloning, DNA amplification, and synthetic methods. Some of the affinity labels and reagents for their detection and isolation are available commercially.

[00212] Various leader sequences known in the art can be used for the efficient secretion of the EphA2 antigenic peptide from bacterial cells such as *Listeria* (von Heijne, 1985, J. *Mol. Biol.* 184:99-105). In addition to the signal sequences described above and in Section 5.1.2.4, suitable leader sequences for targeting EphA2 antigenic peptide expression in bacterial cells include, but are not limited to, the leader sequences of the *E.coli* proteins OmpA (Hobom *et al.*, 1995, *Dev. Biol. Stand.* 84:255-262), Pho A (Oka *et al.*, 1985, *Proc. Natl. Acad. Sci* 82:7212-16), OmpT (Johnson *et al.*, 1996, *Protein Expression* 7:104-113), LamB and OmpF (Hoffman & Wright, 1985, *Proc. Natl. Acad. Sci. USA* 82:5107-5111), β-lactamase (Kadonaga *et al.*, 1984, *J. Biol. Chem.* 259:2149-54), enterotoxins (Morioka-Fujimoto *et al.*, 1991, *J. Biol. Chem.* 266:1728-32), and the *Staphylococcus aureus* protein A (Abrahmsen *et al.*, 1986, *Nucleic Acids Res.* 14:7487-7500), and the *B. subtilis* endoglucanase (Lo *et al.*, *Appl. Environ. Microbiol.* 54:2287-2292), as well as artificial and synthetic signal sequences (MacIntyre *et al.*, 1990, *Mol. Gen. Genet.* 221:466-74; Kaiser *et al.*, 1987, *Science*, 235:312-317).

[00213] In certain embodiments, the fusion partner comprises a non-EphA2 polypeptide corresponding to an antigen associated with the cell type against which a

therapeutic or prophylactic immune is desired. For example, the non-EphA2 polypeptide can comprise an epitope of a tumor-associated antigen, such as, but not limited to, MAGE-1, MAGE-2, MAGE-3, gp100, TRP-2, tyrosinase, MART-1, β-HCG, CEA, Ras, β-catenin, gp43, GAGE-1, GAGE -2, N-acetylglucosaminyltransferase-V, p15, β-catenin, BAGE-1, PSA, MUM-1, CDK4, HER-2/neu, Human papillomavirus-E6, Human papillomavirus-E7, and MUC-1, 2, 3.

[00214] Polynucleotides encoding fusion proteins can be produced by standard recombinant DNA techniques. For example, a nucleic acid molecule encoding a fusion protein can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g., Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, 1992).

[00215] The nucleotide sequence coding for a fusion protein can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The expression of a fusion protein may be regulated by a constitutive, inducible or tissue-specific or -selective promoter. It will be understood by the skilled artisan that fusion proteins, which can facilitate solubility and/or expression, and can increase the *in vivo* half-life of the EphA2 antigenic peptide and thus are useful in the methods of the invention. The EphA2 antigenic peptides or peptide fragments thereof, or fusion proteins can be used in any assay that detects or measures EphA2 antigenic peptides or in the calibration and standardization of such assay.

[00216] The methods of invention encompass the use of EphA2 antigenic peptides or peptide fragments thereof, which may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the EphA2 antigenic peptides of the invention by expressing nucleic acid containing EphA2 antigenic gene sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing, *e.g.*, EphA2 antigenic peptide coding sequences (including but not limited to nucleic acids encoding all or an antigenic portion of a polypeptide of SEQ ID NO:2) and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. See, for example, the techniques described in Sambrook *et al.*, 1989, *supra*, and Ausubel *et al.*, 1989, *supra*.

Alternatively, RNA capable of encoding EphA2 antigenic peptide sequences may be chemically synthesized using, for example, synthesizers (*see, e.g.*, the techniques described in *Oligonucleotide Synthesis*, 1984, Gait, M.J. ed., IRL Press, Oxford).

[00217] In certain embodiments, the EphA2 antigenic peptide is functionally coupled to an internalization signal peptide, also referred to as a "protein transduction domain," that would allow its uptake into the cell nucleus. In certain specific embodiments, the internalization signal is that of Antennapedia (reviewed by Prochiantz, 1996, Curr. Opin. Neurobiol. 6:629-634, Hox A5 (Chatelin *et al.*, 1996, *Mech. Dev.* 55:111-117), HIV TAT protein (Vives *et al.*, 1997, *J. Biol. Chem.* 272:16010-16017) or VP22 (Phelan *et al.*, 1998, *Nat. Biotechnol.* 16:440-443).

5.2.2. POLYNUCLEOTIDES ENCODING EphA2 ANTIGENIC PEPTIDES

[00218] The present invention also encompasses the use of *Listeria*-based vaccines that comprise or contain polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions, *e.g.*, as defined *infra*, to polynucleotides that encode an EphA2 antigenic peptide of the invention.

[00219] By way of example and not limitation, procedures using such conditions of low stringency for regions of hybridization of over 90 nucleotides are as follows (*see also* Shilo and Weinberg, 1981, *Proc. Natl. Acad. Sci. USA* 78:6789-6792). Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and re-exposed to film. Other conditions of low stringency which may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

[00220] Also, by way of example and not limitation, procedures using such conditions of high stringency for regions of hybridization of over 90 nucleotides are as follows. Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP,

0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography.

[00221] Other conditions of high stringency which may be used depend on the nature of the nucleic acid (*e.g.*, length, GC content, *etc.*) and the purpose of the hybridization (detection, amplification, *etc.*) and are well known in the art. For example, stringent hybridization of a nucleic acid of approximately 15-40 bases to a complementary sequence in the polymerase chain reaction (PCR) is done under the following conditions: a salt concentration of 50 mM KCl, a buffer concentration of 10 mM Tris-HCl, a Mg^{2+} concentration of 1.5 mM, a pH of 7-7.5 and an annealing temperature of 55-60°C.

[00222] Selection of appropriate conditions for moderate stringencies is also well known in the art (*see, e.g.*, Sambrook *et al.*, 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; *see also*, Ausubel *et al.*, eds., in the Current Protocols in Molecular Biology series of laboratory technique manuals, © 1987-1997, Current Protocols, © 1994-1997 John Wiley and Sons, Inc.).

[00223] The nucleic acids useful in the present methods may be made by any method known in the art. For example, if the nucleotide sequence of the EphA2 antigenic peptide is known, a nucleic acid encoding the peptide may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the peptide, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[00224] Alternatively, a polynucleotide encoding an EphA2 antigenic peptide may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular peptide is not available, but the sequence of the EphA2 antigenic peptide is known, a nucleic acid encoding the peptide may be chemically synthesized or obtained from a suitable source (*e.g.*, a cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing EphA2) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the peptide.

Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[00225] Further, a nucleic acid that is useful in the present methods may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, *etc.* (see, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate EphA2 antigenic peptides having a different amino acid sequence from the amino acid sequence depicted in SEQ ID NO:2, for example to create amino acid substitutions, deletions, and/or insertions.

5.3. Assays for EphA2 Antigenic Peptides

[00226] The present invention provide *Listeria*-based EphA2 vaccines comprising *Listeria* bacteria engineered to express an EphA2 antigenic peptide. Any assay known in the art for determining whether a peptide is a T cell epitope or a B cell epitope may be employed in testing EphA2 peptides for suitability in the present methods and compositions.

[00227] For example, for determining whether a peptide is a T cell epitope, ELISPOT assays and methods for intracellular cytokine staining can be used for enumeration and characterization of antigen-specific $CD4^+$ and $CD8^+$ T cells. Lalvani *et al.* (1997) *J. Exp. Med.* 186:859-865; Waldrop *et al.* (1997) *J. Clin Invest.* 99:1739-1750.

[00228] EphA2 antigenic peptides can be determined by screening synthetic peptides corresponding to portions of EphA2. Candidate antigenic peptides can be identified on the basis of their sequence or predicted structure. A number of algorithms are available for this purpose.

[00229] Exemplary protocols for such assays are presented below.

5.3.1. Peptides That Display Immunogenicity of EphA2

[00230] The ability of EphA2 peptides to elicit EphA2-specific antibody responses in mammals can be examined, for example, by immunizing animals (*e.g.*, mice, guinea pigs or rabbits) with individual EphA2 peptides emulsified in Freund's adjuvant.

[00231] After three injections (5 to 100 μg peptide per injection), IgG antibody responses are tested by peptide-specific ELISAs and immunoblotting against EphA2.

[00232] EphA2 peptides which produce antisera that react specifically with the EphA2 peptides and also recognized full length EphA2 protein in immunoblots are said to display the antigenicity of EphA2.

5.3.2. CD4$^+$ T-CELL PROLIFERATION ASSAY

[00233] For example, such assays include *in vitro* cell culture assays in which peripheral blood mononuclear cells ("PBMCs") are obtained from fresh blood of a patient with a disease involving overexpression of EphA2, and purified by centrifugation using FICOLL-PLAQUE PLUS (Pharmacia, Upsalla, Sweden) essentially as described by Kruse and Sebald, 1992, *EMBO J.* 11:3237-3244. The peripheral blood mononuclear cells are indubated for 7-10 days with candidate EphA2 antigenic peptides. Antigen presenting cells may optionally be added to the culture 24 to 48 hours prior to the assay, in order to process and present the antigen. The cells are then harvested by centrifugation, and washed in RPMI 1640 media (GibcoBRL, Gaithersburg, MD). 5 x 10^4 activated T cells/well are in RPMI 1640 media containing 10% fetal bovine serum, 10 mM HEPES, ph 7.5, 2 mM L-glutamine, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulphate in 96 well plates for 72 hrs at 37°C, pulsed with 1 μCi ^{3}H-thymidine (DuPont NEN, Boston, MA)/well for 6 hrs, harvested, and radioactivity measured in a TOPCOUNT scintillation counter (Packard Instrument Col., Meriden, CT).

5.3.3. Intracellular Cytokine Staining (ICS)

[00234] Measurement of antigen-specific, intracellular cytokine responses of T cells can be performed essentially as described by Waldrop *et al.*, 1997, *J. Clin. Invest.* 99:1739-1750; Openshaw *et al.*, 1995, *J. Exp. Med.* 182:1357-1367; or Estcourt *et al.*, 1997, *Clin. Immunol. Immunopathol.* 83:60-67. Purified PBMCs from patients with a disease involving EphA2-overexpressing cells are placed in 12x75 millimeter polystyrene tissue culture tubes (Becton Dickinson, Lincoln Park, N.J.) at a concentration of 1x10^6 cells per tube. A solution comprising 0.5 milliliters of HL-1 serum free medium, 100 units per milliliter of penicillin, 100 units per milliliter streptomycin, 2 millimolar L glutamine (Gibco BRL), varying amounts of individual EphA2 antigenic candidate peptides, and 1 unit of anti-CD28 mAb (Becton-Dickinson, Lincoln Park, N.J.) is added to each tube. Anti-CD3 mAb is added to a duplicate set of normal PBMC cultures as positive control. Culture tubes are incubated for 1 hour. Brefeldin A is added to individual tubes at a concentration of 1 microgram per milliliter, and the tubes are incubated for an additional 17 hours.

[00235] PBMCs stimulated as described above are harvested by washing the cells twice with a solution comprising Dulbecco's phosphate-buffered saline (dPBS) and 10 units

of Brefeldin A. These washed cells are fixed by incubation for 10 minutes in a solution comprising 0.5 milliliters of 4% paraformaldehyde and dPBS. The cells are washed with a solution comprising dPBS and 2% fetal calf serum (FCS). The cells are then either used immediately for intracellular cytokine and surface marker staining or are frozen for no more than three days in freezing medium, as described (Waldrop *et al.*, 1997, *J. Clin. Invest.* 99:1739-1750).

[00236] The cell preparations were rapidly thawed in a 37°C water bath and washed once with dPBS. Cells, either fresh or frozen, are resuspended in 0.5 milliliters of permeabilizing solution (Becton Dickinson Immunocytometry systems, San Jose, Calif.) and incubated for 10 minutes at room temperature with protection from light. Permeabilized cells are washed twice with dPBS and incubated with directly conjugated mAbs for 20 minutes at room temperature with protection from light. Optimal concentrations of antibodies are predetermined according to standard methods. After staining, the cells were washed, refixed by incubation in a solution comprising dPBS 1% paraformaldehyde, and stored away from light at 4°C for flow cytometry analysis.

5.3.4. ELISPOT Assays

[00237] The ELISPOT assay measures Th1-cytokine specific induction in murine splenocytes following *Listeria* vaccination. ELISPOT assays are performed to determine the frequency of T lymphocytes in response to endogenous antigenic peptide stimulation, and are as described in Geginat *et al.*, 2001, *J. Immunol.* 166:1877-1884. Balb/c mice (3 per group) are vaccinated with *L. monocytogenes* expressing candidate EphA2 antigenic peptides or HBSS as control. Whole mouse spleens are harvested and pooled five days after vaccination. Single cell suspensions of murine splenocytes are plated in the presence of various antigens overnight in a 37°C incubator.

[00238] Assays are performed in nitrocellulose-backed 96-well microtiter plates coated with rat anti-mouse IFN-γ mAb. For the testing of the candidate EphA2 antigenic peptide, a 1 x 10^{-5} M peptide solution is prepared. In round-bottom 96-well microtiter plates per well 6 x 10^{5} unseparated splenocytes in 135 µl culture medium (a modification of Eagle's medium (Life Technologies, Eggenstein, Germany) supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 x 10^{-5} M 2-ME, and 2 mM glutamine) are mixed with 15 µl of the 1 x 10^{-5} M peptide solution to yield a final peptide concentration of 1 x 10^{-6} M. After 6 h of incubation at 37°C, cells are resuspended by vigorous pipetting, and 100 µl or 10 µl of cell suspension (4 x 10^{5}/well or 4 x 10^{4}/well, respectively) is transferred to Ab-coated ELISPOT plates and incubated overnight at 37°C. In the

ELISPOT plates, the final volume was adjusted to 150 μl to ensure homogenous distribution of cells.

[00239] Purified $CD4^+$ or $CD8^+$ T cells are tested in a modified assay as follows: 15 μl prediluted peptide (1×10^{-5} M) is directly added to Ab-coated ELISPOT plates and mixed with 4×10^5 splenocytes from nonimmune animals as APC to yield a final volume of 100 μl. After 4 h of preincubation of APC at 37°C, 1×10^5 $CD4^+$ or $CD8^+$ cells purified from *L. monocytogenes*-immune mice are added per well in a volume of 50 μl and plates are incubated overnight at 37°C. The ELISPOT-based *ex vivo* MHC restriction analysis is performed after loading of cell lines expressing specific MHC class I molecules with 1×10^{-6} M peptide for 2 h at 37°C. Subsequently, unbound peptides are washed off (four times) to prevent binding of peptides to responder splenocytes. Per well of the ELISPOT plate, 1×10^5 peptide-loaded APC are mixed with 4×10^5 or 4×10^4 responder splenocytes in a final volume of 150 μl. After overnight incubation at 37°C, ELISPOT plates are developed with biotin-labeled rat anti-mouse IFN- γ mAb, HRP streptavidin conjugate, and aminoethylcarbazole dye of spots per splenocytes seeded. The specificity and sensitivity of the ELISPOT assay is controlled with IFN- γ secreting CD8 T cell lines specific for a control antigen.

5.4. Prophylactic/Therapeutic Methods

[00240] The present invention provides methods for treating, preventing, or managing a disorder associated with overexpression of EphA2 and/or hyperproliferative cell disorders, preferably cancer, comprising administering to a subject in need thereof one or more *Listeria*-based EphA2 vaccines of the invention.

[00241] The present invention encompasses methods for eliciting an immune response against an EphA2-expressing cell associated with a hyperproliferative cell disorder, comprising administering to a subject one or more *Listeria*-based EphA2 vaccines of the invention in an amount effective for eliciting an immune response against the EphA2-expressing cell.

[00242] In another specific embodiment, the disorder to be treated, prevented, or managed is a pre-cancerous condition associated with cells that overexpress EphA2. In more specific embodiments, the pre-cancerous condition is high-grade prostatic intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or compound nevi.

[00243] The present invention provides methods for treating, preventing, or managing a disorder associated with overexpression of EphA2 and/or hyperproliferative cell disorders, preferably cancer, comprising administering to a subject in need thereof one

or more *Listeria*-based EphA2 vaccines of the invention and one or more other therapies. Examples of other therapies include, but are not limited to, those listed below in Section 5.4.3, *infra*. In one embodiment, the *Listeria*-based EphA2 vaccine of the invention can be administered in combination with one or more other therapies (*e.g.*, prophylactic or therapeutic agents) useful in the treatment, prevention or management of disorders associated with EphA2 overexpression and/or hyperproliferative cell disorders, such as cancer. In certain embodiments, one or more *Listeria*-based EphA2 vaccines are administered to a subject, preferably a human, concurrently with one or more other therapies (*e.g.*, therapeutic agents) useful for the treatment or management of cancer. The term "concurrently" is not limited to the administration of therapies (*e.g.*, prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a *Listeria*-based EphA2 vaccine of the invention and another therapy are administered to a subject in a sequence and within a time interval such that the *Listeria*-based EphA2 vaccine can act together with the other therapy to provide an increased benefit than if they were administered otherwise. For example, each therapy (*e.g.*, prophylactic or therapeutic agent) may be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy (*e.g.*, prophylactic or therapeutic agent) can be administered separately, in any appropriate form and by any suitable route. In certain embodiments, the *Listeria*-based EphA2 vaccines of the invention are administered before, concurrently or after surgery. Preferably, the surgery completely removes localized tumors or reduces the size of large tumors. Surgery can also be done as a preventive measure or to relieve pain.

[00244] In various embodiments, the therapies (*e.g.*, prophylactic or therapeutic agents) are administered less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In preferred embodiments, two or more therapies are administered within the same patient visit.

[00245] The dosage amounts and frequencies of administration provided herein are encompassed by the terms therapeutically effective and prophylactically effective. The dosage and frequency further will typically vary according to factors specific for each

patient depending on the specific therapeutic or prophylactic agents administered, the severity and type of cancer, the route of administration, as well as age, body weight, response, and the past medical history of the patient. Suitable regimens can be selected by one skilled in the art by considering such factors and by following, for example, dosages reported in the literature and recommended in the *Physician's Desk Reference* (56^{th} ed., 2002, 57^{th} ed., 2003 and 58^{th} ed., 2004).

5.4.1.1. <u>Patient Population</u>

[00246] The invention provides methods for treating, preventing, and/or managing a disorder associated with EphA2 overexpression and/or hyperproliferative cell disease, particularly cancer, comprising administrating to a subject in need thereof one or more *Listeria*-based EphA2 vaccines of the invention in a therapeutically or prophylactically effective amount or an amount effective to elicit an immune response against EphA2-expressing cells associated with the hyperproliferative disorder. In another embodiment, an effective amount of a *Listeria*-based EphA2 vaccine of the invention is administered in combination with an effective amount of one or more other therapies (*e.g.*, therapeutic or prophylactic agents) to treat, prevent, and/or manage a disorder associated with EphA2 overexpression and/or hyperproliferative cell disease, particularly cancer. The subject is preferably a mammal such as non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats, *etc.*) and a primate (*e.g.*, monkey, such as a cynomolgous monkey and a human). In a preferred embodiment, the subject is a human.

[00247] Specific examples of cancers that can be treated by the methods encompassed by the invention include, but are not limited to, cancers that overexpress EphA2. In one embodiment, the cancer is of an epithelial origin. Examples of such cancers are cancer of the lung, colon, prostate, breast, and skin. Other cancers include cancer of the bladder and pancreas and renal cell carcinoma and melanoma. In another embodiment, the cancer is a solid tumor. In another embodiment, the cancer is of a T cell origin. Examples of such cancers are leukemias and lymphomas. Additional cancers are listed by example and not by limitation in the following Section 5.4.1.1. In particular embodiments, methods of the invention can be used to treat and/or prevent metastasis from primary tumors.

[00248] The methods and compositions of the invention comprise the administration of one or more *Listeria*-based EphA2 vaccines of the invention to subjects/patients suffering from or expected to suffer from cancer, *e.g.*, have a genetic predisposition for a particular type of cancer, have been exposed to a carcinogen, or are in remission from a particular cancer. As used herein, "cancer" refers to primary or metastatic cancers. Such patients may or may not have been previously treated for cancer. The methods and

compositions of the invention may be used as any line of cancer therapy, e.g., a first line, second line or third line of cancer therapy. Included in the invention is also the treatment of patients undergoing other cancer therapies and the methods and compositions of the invention can be used before any adverse effects or intolerance of these other cancer therapies occurs. The invention also encompasses methods for administering one or more *Listeria*-based EphA2 vaccines of the invention to treat or ameliorate symptoms in refractory patients. In a certain embodiment, that a cancer is refractory to a therapy means that at least some significant portion of the cancer cells are not killed or their cell division arrested. The determination of whether the cancer cells are refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a cancer is refractory where the number of cancer cells has not been significantly reduced, or has increased. The invention also encompasses methods for administering one or more *Listeria*-based EphA2 vaccines to prevent the onset or recurrence of cancer in patients predisposed to having cancer.

[00249] In particular embodiments, the *Listeria*-based EphA2 vaccines of the invention are administered to reverse resistance or reduced sensitivity of cancer cells to certain hormonal, radiation and chemotherapeutic agents thereby resensitizing the cancer cells to one or more of these agents, which can then be administered (or continue to be administered) to treat or manage cancer, including to prevent metastasis. In a specific embodiment, the *Listeria*-based EphA2 vaccines of the invention are administered to patients with increased levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy. In another specific embodiment, the *Listeria*-based EphA2 vaccines of the invention are administered to patients suffering from breast cancer that have a decreased responsiveness or are refractory to tamoxifen treatment. In another specific embodiment, the *Listeria*-based EphA2 vaccines of the invention are administered to patients with increased levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy.

[00250] In alternate embodiments, the invention provides methods for treating or managing a patients' cancer comprising administering to the patient one or more *Listeria*-based EphA2 vaccines of the invention in combination with any other therapy or to patients who have proven refractory to other therapies but are no longer on these therapies. In certain embodiments, the patients being treated by the methods of the invention are patients

already being treated with chemotherapy, radiation therapy, hormonal therapy, or biological therapy/immunotherapy. Among these patients are refractory patients and those with cancer despite treatment with existing cancer therapies. In other embodiments, the patients have been treated and have no disease activity and one or more Listeria-based EphA2 vaccines of the invention are administered to prevent the recurrence of cancer.

[00251] In preferred embodiments, the existing therapy is chemotherapy. In particular embodiments, the existing therapy includes administration of chemotherapies including, but not limited to, methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, etoposides, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, docetaxel, etc. Among these patients are patients treated with radiation therapy, hormonal therapy and/or biological therapy/immunotherapy. Also among these patients are those who have undergone surgery for the treatment or management of cancer.

[00252] Alternatively, the invention also encompasses methods for treating or managing patients undergoing or having undergone radiation therapy. Among these are patients being treated or previously treated with chemotherapy, hormonal therapy and/or biological therapy/immunotherapy. Also among these patients are those who have undergone surgery for the treatment of cancer.

[00253] In other embodiments, the invention encompasses methods for treating patients undergoing or having undergone hormonal therapy and/or biological therapy/immunotherapy. Among these are patients being treated or having been treated with chemotherapy and/or radiation therapy. Also among these patients are those who have undergone surgery for the treatment of cancer.

[00254] Additionally, the invention also provides methods of treatment or management of cancer as an alternative to chemotherapy, radiation therapy, hormonal therapy, and/or biological therapy/immunotherapy where the therapy has proven or may prove too toxic, *i.e.*, results in unacceptable or unbearable side effects, for the subject being treated. The subject being treated with the methods of the invention may, optionally, be treated with other cancer therapies such as surgery, chemotherapy, radiation therapy, hormonal therapy or biological therapy, depending on which therapy was found to be unacceptable or unbearable.

[00255] In other embodiments, the invention provides administration of one or more *Listeria*-based EphA2 vaccines of the invention without any other cancer therapies for the

treatment of cancer, but who have proved refractory to such treatments. In specific embodiments, patients refractory to other cancer therapies are administered one or more EphA2 vaccines in the absence of cancer therapies.

[00256] In other embodiments, patients with a pre-cancerous condition associated with cells that overexpress EphA2 can be administered vaccines of the invention to treat the disorder and decrease the likelihood that it will progress to malignant cancer. In a specific embodiments, the pre-cancerous condition is high-grade prostatic intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or compound nevi.

[00257] In yet other embodiments, the invention provides methods of treating, preventing and/or managing hyperproliferative cell disorders other than cancer, particularly those associated with overexpression of EphA2, including but not limited to, asthma, chromic obstructive pulmonary disorder (COPD), fibrosis (*e.g.*, lung, kidney, heart and liver fibrosis), restenosis (smooth muscle and/or endothelial), psoriasis, etc.. These methods include methods analogous to those described above for treating, preventing and managing cancer, for example, by administering the EphA2 vaccines of the invention, combination therapy (see, *e.g.*, Section 5.4.3, *infra*, for examples of other therapies to administer in combination with the EphA2 vaccines to a subject to treat, prevent or manage a hyperproliferative disorder other than cancer), administration to patients refractory to particular treatments, *etc.*

5.4.1.2. Cancers

[00258] Cancers and related disorders that can be treated, prevented, or managed by methods and compositions of the present invention include but are not limited to cancers of an epithelial cell origin and/or an endothelial origin. Examples of such cancers include the following: leukemias, such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias, such as, myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia leukemias and myelodysplastic syndrome; chronic leukemias, such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenström's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as but not limited to bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone,

chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, synovial sarcoma; brain tumors such as but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, primary brain lymphoma; breast cancer including but not limited to ductal carcinoma, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, and inflammatory breast cancer; adrenal cancer such as but not limited to pheochromocytom and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer such as but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius; eye cancers such as but not limited to ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma; vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers such as but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited to endometrial carcinoma and uterine sarcoma; ovarian cancers such as but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers such as but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers such as but not limited to hepatocellular carcinoma and hepatoblastoma; gallbladder cancers such as adenocarcinoma; cholangiocarcinomas such as but not limited to papillary, nodular, and diffuse; lung cancers such as non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, prostatic

intraepithelial neoplasia, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal cancers; oral cancers such as but not limited to squamous cell carcinoma; basal cancers; salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and verrucous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers such as but not limited to renal cell carcinoma, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/ or uterer); Wilms' tumor; bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy et al., 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin Books U.S.A., Inc., United States of America)

[00259] The methods and compositions of the invention are also useful in the treatment or prevention of a variety of cancers or other abnormal proliferative diseases, including (but not limited to) the following: carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin; including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T cell lymphoma, Burkitt's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscarama, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer and teratocarcinoma. It is also contemplated that cancers caused by aberrations in apoptosis would also be treated by the methods and compositions of the invention. Such cancers may include but not be limited to follicular lymphomas, carcinomas with p53 mutations, hormone dependent tumors of the

breast, prostate and ovary, and precancerous lesions such as familial adenomatous polyposis, and myelodysplastic syndromes. In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated or prevented in the skin, lung, colon, breast, prostate, bladder, kidney, pancreas, ovary, or uterus. In other specific embodiments, sarcoma, melanoma, or leukemia is treated or prevented.

[00260] In some embodiments, the cancer is malignant and overexpresses EphA2. In other embodiments, the disorder to be treated is a pre-cancerous condition associated with cells that overexpress EphA2. In a specific embodiments, the pre-cancerous condition is high-grade prostatic intraepithelial neoplasia (PIN), fibroadenoma of the breast, fibrocystic disease, or compound nevi.

[00261] In preferred embodiments, the methods and compositions of the invention are used for the treatment and/or prevention of breast, ovarian, esophageal, colon, ovarian, lung, and prostate cancers and melanoma and are provided below by example rather than by limitation.

[00262] In another preferred embodiment, the methods and compositions of the invention are used for the treatment and/or prevention of cancers of T cell origin, including, but not limited to, leukemias and lymphomas.

5.4.1.3. Treatment of Breast Cancer

[00263] In specific embodiments, patients with breast cancer are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for breast cancer therapy including but not limited to: doxorubicin, epirubicin, the combination of doxorubicin and cyclophosphamide (AC), the combination of cyclophosphamide, doxorubicin and 5-fluorouracil (CAF), the combination of cyclophosphamide, epirubicin and 5-fluorouracil (CEF), her-2 antibodies, e.g., herceptin, tamoxifen, the combination of tamoxifen and cytotoxic chemotherapy, taxanes (such as docetaxel and paclitaxel). In a further embodiment, peptides of the invention can be administered with taxanes plus standard doxorubicin and cyclophosphamide for adjuvant treatment of node-positive, localized breast cancer.

[00264] In a specific embodiment, patients with pre-cancerous fibroadenoma of the breast or fibrocystic disease are administered a *Listeria*-based EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant breast cancer. In another specific embodiment. patients refractory to treatment, particularly

hormonal therapy, more particularly tamoxifen therapy, are administered a *Listeria*-based EphA2 vaccine of the invention to treat the cancer and/or render the patient non-refractory or responsive.

5.4.1.4. Treatment of Colon Cancer

[00265] In specific embodiments, patients with colon cancer are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for colon cancer therapy including but not limited to: AVASTIN™ (bevacizumab), the combination of 5-FU and leucovorin, the combination of 5-FU and levamisole, irinotecan (CPT-11) or the combination of irinotecan, 5-FU and leucovorin (IFL).

5.4.1.5. Treatment of Prostate Cancer

[00266] In specific embodiments, patients with prostate cancer are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for prostate cancer therapy including but not limited to: external-beam radiation therapy, interstitial implantation of radioisotopes (*i.e.*, I^{125}, palladium, iridium), leuprolide or other LHRH agonists, non-steroidal antiandrogens (flutamide, nilutamide, bicalutamide), steroidal antiandrogens (cyproterone acetate), the combination of leuprolide and flutamide, estrogens such as DES, chlorotrianisene, ethinyl estradiol, conjugated estrogens U.S.P., DES-diphosphate, radioisotopes, such as strontium-89, the combination of external-beam radiation therapy and strontium-89, second-line hormonal therapies such as aminoglutethimide, hydrocortisone, flutamide withdrawal, progesterone, and ketoconazole, low-dose prednisone, or other chemotherapy regimens reported to produce subjective improvement in symptoms and reduction in PSA level including docetaxel, paclitaxel, estramustine/docetaxel, estramustine/etoposide, estramustine/vinblastine, and estramustine/paclitaxel.

[00267] In a specific embodiment, patients with pre-cancerous high-grade prostatic intraepithelial neoplasia (PIN) are administered an EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant prostate cancer.

5.4.1.6. Treatment of Melanoma

[00268] In specific embodiments, patients with melanoma are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with

an effective amount of one or more other agents useful for melanoma cancer therapy including but not limited to: dacarbazine (DTIC), nitrosoureas such as carmustine (BCNU) and lomustine (CCNU), agents with modest single agent activity including vinca alkaloids, platinum compounds, and taxanes, the Dartmouth regimen (cisplatin, BCNU, and DTIC), interferon alpha (IFN-α), and interleukin-2 (IL-2). In a specific embodiment, an effective amount of one or more EphA2 vaccines of the invention can be administered in combination with isolated hyperthermic limb perfusion (ILP) with melphalan (L-PAM), with or without tumor necrosis factor-alpha (TNF-α) to patients with multiple brain metastases, bone metastases, and spinal cord compression to achieve symptom relief and some shrinkage of the tumor with radiation therapy.

[00269] In a specific embodiment, patients with pre-cancerous compound nevi are administered a *Listeria*-based EphA2 vaccine of the invention to treat the disorder and decrease the likelihood that it will progress to malignant melanoma.

5.4.1.7. Treatment of Ovarian Cancer

[00270] In specific embodiments, patients with ovarian cancer are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in combination with an effective amount of one or more other agents useful for ovarian cancer therapy including but not limited to: intraperitoneal radiation therapy, such as P^{32} therapy, total abdominal and pelvic radiation therapy, cisplatin, the combination of paclitaxel (Taxol) or docetaxel (Taxotere) and cisplatin or carboplatin, the combination of cyclophosphamide and cisplatin, the combination of cyclophosphamide and carboplatin, the combination of 5-FU and leucovorin, etoposide, liposomal doxorubicin, gemcitabine or topotecan. It is contemplated that an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention is administered in combination with the administration Taxol for patients with platinum-refractory disease. Included is the treatment of patients with refractory ovarian cancer including administration of: ifosfamide in patients with disease that is platinum-refractory, hexamethylmelamine (HMM) as salvage chemotherapy after failure of cisplatin-based combination regimens, and tamoxifen in patients with detectable levels of cytoplasmic estrogen receptor on their tumors.

5.4.1.8. Treatment of Lung Cancers

[00271] In specific embodiments, patients with small lung cell cancer are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention. In another embodiment, the peptides of the invention can be administered in

combination with an effective amount of one or more other agents useful for lung cancer therapy including but not limited to: thoracic radiation therapy, cisplatin, vincristine, doxorubicin, and etoposide, alone or in combination, the combination of cyclophosphamide, doxorubicin, vincristine/etoposide, and cisplatin (CAV/EP), local palliation with endobronchial laser therapy, endobronchial stents, and/or brachytherapy.

[00272] In other specific embodiments, patients with non-small lung cell cancer are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention in combination with an effective amount of one or more other agents useful for lung cancer therapy including but not limited to: palliative radiation therapy, the combination of cisplatin, vinblastine and mitomycin, the combination of cisplatin and vinorelbine, paclitaxel, docetaxel or gemcitabine, the combination of carboplatin and paclitaxel, interstitial radiation therapy for endobronchial lesions or stereotactic radiosurgery.

5.4.1.9. Treatment of T cell Malignancies

[00273] In specific embodiments, patients with T cell malignancies, such as leukemias and lymphomas (*see*, *e.g.*, section 5.8.1.1), are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention. In another embodiment, the EphA2 vaccines of the invention can be administered in combination with an effective amount of one or more other agents useful for the prevention, treatment or amelioration of cancer, particularly T cell malignancies or one or more symptoms thereof, said combination therapies comprising administering to a subject in need thereof a prophylactically or therapeutically effective amount of one or more *Listeria*-based EphA2 vaccines of the invention and a prophylactically or therapeutically effective amount of one or more cancer therapies, including chemotherapies, hormonal therapies, biological therapies, immunotherapies, or radiation therapies.

[00274] In another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention in combination with one or more cancer chemotherapeutic agents, such as but not limited to: doxorubicin, epirubicin, cyclophosphamide, 5-fluorouracil, taxanes such as docetaxel and paclitaxel, leucovorin, levamisole, irinotecan, estramustine, etoposide, vinblastine, dacarbazine, nitrosoureas such as carmustine and lomustine, vinca alkaloids, platinum compounds, cisplatin, mitomycin, vinorelbine, gemcitabine, carboplatin, hexamethylmelamine and/or topotecan. Such methods can optionally further comprise the administration of other cancer therapies, such as but not limited to radiation therapy, biological therapies, hormonal therapies and/or surgery.

[00275] In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention in combination with one or more types of radiation therapy, such as external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, palladium, iridium), radioisotopes such as strontium-89, thoracic radiation therapy, intraperitoneal P-32 radiation therapy, and/or total abdominal and pelvic radiation therapy. Such methods can optionally further comprise the administration of other cancer therapies, such as but not limited to chemotherapies, biological therapies/immunotherapies, hormonal therapies and/or surgery.

[00276] In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention in combination with one or more biological therapies/immunotherapies or hormonal therapies, such as tamoxifen, leuprolide or other LHRH agonists, non-steroidal antiandrogens (flutamide, nilutamide, bicalutamide), steroidal antiandrogens (cyproterone acetate), estrogens (DES, chlorotrianisene, ethinyl estradiol, congugated estrogens U.S.P., DES-diphosphate), aminoglutethimide, hydrocortisone, flutamide withdrawal, progesterone, ketoconazole, prednisone, interferon-α, interleukin-2, tumor necrosis factor-α, and/or melphalan. Biological therapies also included are cytokines such as but not limited to TNF ligand family members such as TRAIL anti-cancer agonists that induce apoptosis, TRAIL antibodies that bind to TRAIL receptors 1 and 2 otherwise known as DR4 and DR5 (Death Domain Containing Receptors 4 and 5), as well as DR4 and DR5. TRAIL and TRAIL antibodies, ligands and receptors are known in the art and described in U.S. Patent Nos. 6,342,363, 6,284,236, 6,072,047 and 5,763,223. Such methods can optionally further comprise the administration of other cancer therapies, such as but not limited to radiation therapy, chemotherapies, and/or surgery.

[00277] In yet another specific embodiment, patients with T cell malignancies are administered an effective amount of one or more *Listeria*-based EphA2 vaccines of the invention in combination with standard and experimental therapies of T cell malignancies. Standard and experimental therapies of T cell malignancies that can be used in the methods and compositions of the invention include, but are not limited to, antibody therapy (*e.g.*, Campath®, anti-Tac, HuM291 (humanized murine IgG2 monoclonal antibody against CD3), antibody drug conjugates (*e.g.*, Mylotarg), radiolabeled monoclonal antibodies (*e.g.*, Bexxar, Zevalin, Lym-1)), cytokine therapy, aggressive combination chemotherapy with or without cytotoxic agents, purine analogs, hematopoietic stem cell transplantation, and T cell mediated therapy (*e.g.*, CD8+ T cells with anti-leukemic activity against target antigens

including but not limited to leukemia specific proteins (*e.g.*, bcr/abl, PML/RARa, EMV/AML-1), leukemia-associated proteins (*e.g.*, proteinase 3, WT-1, h-TERT, hdm-2)). (*See* Riddell *et el.*, 2002, *Cancer Control*, 9(2): 114-122; Dearden *et al.* , 2002, *Medical Oncology*, 19, Suppl. S27-32; Waldmann *et al.* 2000, *Hematology (Am Soc Hematol Educ Program)*:394-408).

5.4.2. Treatment or Prevention of Disorders Associated with Aberrant Angiogenesis

[00278] EphA2 is as a marker of angiogenic blood vessels and plays a critical role in angiogenesis or neovascularization (see, *e.g.*, Ogawa *et al.*, 2000, Oncogene. 19(52):6043-52; Hess *et al.*, 2001, Cancer Res. 61(8):3250-5). Angiogenesis is characterized by the invasion, migration and proliferation of smooth muscle and endothelial cells. The growth of new blood vessels, or angiogenesis, contributes to pathological conditions such as diabetic retinopathy (Adonis *et al.*, 1994, Amer. J. Ophthal., 118:445), rheumatoid arthritis (Peacock *et al.*, 1992, J. Exp. Med., 175:1135) and osteoarthritis (Ondrick *et al.*, 1992, Clin.-Podiatr.-Med.-Surg. 9:185).

[00279] The *Listeria*-based compositions of the invention may therefore be administered to a subject in need thereof to prevent, manage, treat or ameliorate a disorder associated with aberrant angiogenesis or one or more symptoms thereof.

[00280] Disorders that are associated with or characterized by aberrant angiogenesis and may be prevented, treated, managed, or ameliorated with the *Listeria*-based compositions of the invention include, but are not limited to, neoplastic diseases (non-limiting examples are metastases of tumors and leukemia); diseases of ocular neovascularization (non-limiting examples are age-related macular degeneration, diabetic retinopathy, and retinopathy of prematurity, vascular restenosis); skin diseases (non-limiting examples are infantile hemangiomas, verruca vulgaris, psoriasis, basal cell and squamous cell carcinomas, cutaneous melanoma, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa); arthritis (non-limiting examples are rheumatoid arthritis, ankylosing spondylitis, systemic lupus, psoriatic arthropathy, Reiter's syndrome, and Sjogren's syndrome); gynecologic diseases (non-limiting examples are endometriosis, preeclampsia during pregnancy, carcinoma of the ovary, endometrium and cervix); and cardiovascular diseases (non-limiting examples are formation of atherosclerotic plaques, atherosclerosis and coronary artery disease).

[00281] In specific embodiments, the disorders that are associated with or characterized by aberrant angiogenesis and that may be prevented, treated, managed, or ameliorated with the *Listeria*-based compositions of the invention include chronic articular

rheumatism, psoriasis, diabetic retinopathy, neovascular glaucoma, macular degeneration, capillary proliferation in atherosclerotic plaques as well as cancers in which EphA2 is expressed in the vasculature. Such cancer disorders can include, for example, solid tumors, tumor metastasis, angiofibromas, retrolental, fibroplasia, hemangiomas, Kaposi's sarcoma.

[00282] In certain embodiments, the *Listeria*-based compositions are employed in combination therapy regimens involving other therapies. Non-limiting examples of such therapies include analgesics, angiogenesis inhibitors, anti-cancer therapies and anti-inflammatory agents, in particular analgesics and angiogenesis inhibitors.

5.4.2.1. Patient Population

[00283] The present invention encompasses methods for treating, managing, or preventing a disorder associated with aberrant angiogenesis or a symptom thereof, in a subject comprising administering one or more *Listeria*-based EphA2 vaccines. The methods of the invention comprise the administration of one or more *Listeria*-based EphA2 vaccines to patients suffering from or expected to suffer from (*e.g.*, patients with a genetic predisposition for or patients that have previously suffered from) a disorder associated with aberrant angiogenesis. Such patients may have been previously treated or are currently being treated for the disorder. In accordance with the invention, a *Listeria*-based EphA2 vaccine may be used as any line of therapy, including, but not limited to, a first, second, third and fourth line of therapy. Further, in accordance with the invention, a *Listeria*-based EphA2 vaccine can be used before any adverse effects or intolerance of the *Listeria*-based EphA2 vaccine therapies occurs. The invention encompasses methods for administering one or more *Listeria*-based EphA2 vaccines of the invention to prevent the onset or recurrence of a disorder associated with aberrant angiogenesis.

[00284] In one embodiment, the invention also provides methods of treatment or management of a disorder associated with aberrant angiogenesis as alternatives to current therapies. In a specific embodiment, the current therapy has proven or may prove too toxic (*i.e.*, results in unacceptable or unbearable side effects) for the patient. In another embodiment, the patient has proven refractory to a current therapy. In such embodiments, the invention provides for the administration of one or more *Listeria*-based EphA2 vaccines of the invention without any other therapies for treating or managing the disorder associated with aberrant angiogenesis. In certain embodiments, one or more *Listeria*-based EphA2 vaccines of the invention can be administered to a patient in need thereof instead of another therapy to treat or manage a disorder associated with aberrant angiogenesis.

[00285] The present invention also encompasses methods for administering one or more *Listeria*-based EphA2 vaccines of the invention to treat or ameliorate symptoms of a

disorder associated with aberrant angiogenesis in patients that are or have become refractory to non-*Listeria*-based EphA2 vaccine therapies. The determination of whether the symptoms are refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a therapy on affected cells in the disorder associated with aberrant angiogenesis, or in patients that are or have become refractory to non-*Listeria*-based EphA2 vaccine therapies.

5.4.3. <u>Other Therapies</u>

[00286] In some embodiments, therapy by administration of one or more *Listeria*-based EphA2 vaccines is combined with the administration of one or more therapies such as, but not limited to, chemotherapies, radiation therapies, hormonal therapies, and/or biological therapies/immunotherapies. Prophylactic/therapeutic agents include, but are not limited to, proteinaceous molecules, including, but not limited to, peptides, polypeptides, proteins, including post-translationally modified proteins, peptides etc.; or small molecules (less than 1000 daltons), inorganic or organic compounds; or nucleic acid molecules including, but not limited to, double-stranded or single-stranded DNA, or double-stranded or single-stranded RNA, as well as triple helix nucleic acid molecules. Prophylactic/therapeutic agents can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi, and protista, or viruses) or from a library of synthetic molecules.

[00287] In a specific embodiment, the methods of the invention encompass administration of a *Listeria*-based EphA2 vaccine of the invention in combination with the administration of one or more prophylactic/therapeutic agents, including antibodies, that are inhibitors of kinases such as, but not limited to, ABL, ACK, AFK, AKT (*e.g.*, AKT-1, AKT-2, and AKT-3), ALK, AMP-PK, ATM, Auroral, Aurora2, bARK1, bArk2, BLK, BMX, BTK, CAK, CaM kinase, CDC2, CDK, CK, COT, CTD, DNA-PK, EGF-R, ErbB-1, ErbB-2, ErbB-3, ErbB-4, ERK (*e.g.*, ERK1, ERK2, ERK3, ERK4, ERK5, ERK6, ERK7), ERT-PK, FAK, FGR (*e.g.*, FGF1R, FGF2R), FLT (*e.g.*, FLT-1, FLT-2, FLT-3, FLT-4), FRK, FYN, GSK (*e.g.*, GSK1, GSK2, GSK3-alpha, GSK3-beta, GSK4, GSK5), G-protein coupled receptor kinases (GRKs), HCK, HER2, HKII, JAK (*e.g.*, JAK1, JAK2, JAK3, JAK4), JNK (*e.g.*, JNK1, JNK2, JNK3), KDR, KIT, IGF-1 receptor, IKK-1, IKK-2, INSR (insulin receptor), IRAK1, IRAK2, IRK, ITK, LCK, LOK, LYN, MAPK, MAPKAPK-1, MAPKAPK-2, MEK, MET, MFPK, MHCK, MLCK, MLK3, NEU, NIK, PDGF receptor alpha, PDGF receptor beta, PHK, PI-3 kinase, PKA, PKB, PKC, PKG, PRK1, PYK2, p38 kinases, p135tyk2, p34cdc2, p42cdc2, p42mapk, p44mpk, RAF, RET, RIP, RIP-2, RK, RON, RS kinase, SRC, SYK, S6K, TAK1, TEC, TIE1, TIE2, TRKA, TXK, TYK2, UL13,

VEGF, VEGFR1, VEGFR2, YES, YRK, ZAP-70, and all subtypes of these kinases (see *e.g.*, Hardie and Hanks (1995) The Protein Kinase Facts Book, I and II, Academic Press, San Diego, Calif.). In preferred embodiments, a *Listeria*-based EphA2 vaccine of the invention is administered in combination with the administration of one or more prophylactic/therapeutic agents that are inhibitors of Eph receptor kinases (*e.g.*, EphA2, EphA4). In a most preferred embodiment, an EphA2 vaccine of the invention is administered in combination with the administration of one or more prophylactic/therapeutic agents that are inhibitors of EphA2.

[00288] In a specific embodiment, the methods of the invention encompass administration of a *Listeria*-based EphA2 vaccine of the invention in combination with the administration of one or more therapeutic antibodies. Examples of therapeutic antibodies that can be used in methods of the invention include but are not limited to AVASTIN® which is an anti-VEGF antibody; antibodies that immunospecifically bind to EphA2 induce signal transduction (*i.e.*, EphA2 agonistic antibodies); antibodies that immunospecifically bind to EphrinA1; HERCEPTIN® (Trastuzumab) (Genentech, CA) which is a humanized anti-HER2 monoclonal antibody for the treatment of patients with metastatic breast cancer; REOPRO® (abciximab) (Centocor) which is an anti-glycoprotein IIb/IIIa receptor on the platelets for the prevention of clot formation; ZENAPAX® (daclizumab) (Roche Pharmaceuticals, Switzerland) which is an immunosuppressive, humanized anti-CD25 monoclonal antibody for the prevention of acute renal allograft rejection; PANOREX™ which is a murine anti-17-IA cell surface antigen IgG2a antibody (Glaxo Wellcome/Centocor); BEC2 which is a murine anti-idiotype (GD3 epitope) IgG antibody (ImClone System); IMC-C225 which is a chimeric anti-EGFR IgG antibody (ImClone System); VITAXIN™ which is a humanized anti-$\alpha_v\beta_3$ integrin antibody (Applied Molecular Evolution/MedImmune); Campath 1H/LDP-03 which is a humanized anti CD52 IgG1 antibody (Leukosite); Smart M195 which is a humanized anti-CD33 IgG antibody (Protein Design Lab/Kanebo); RITUXAN™ which is a chimeric anti-CD20 IgG1 antibody (IDEC Pharm/Genentech, Roche/Zettyaku); LYMPHOCIDE™ which is a humanized anti-CD22 IgG antibody (Immunomedics); LYMPHOCIDE™ Y-90 (Immunomedics); Lymphoscan (Tc-99m-labeled; radioimaging; Immunomedics); Nuvion (against CD3; Protein Design Labs); CM3 is a humanized anti-ICAM3 antibody (ICOS Pharm); IDEC-114 is a primatied anti-CD80 antibody (IDEC Pharm/Mitsubishi); ZEVALIN™ is a radiolabelled murine anti-CD20 antibody (IDEC/Schering AG); IDEC-131 is a humanized anti-CD40L antibody (IDEC/Eisai); IDEC-151 is a primatized anti-CD4 antibody (IDEC); IDEC-152 is a primatized anti-CD23 antibody (IDEC/Seikagaku); SMART anti-CD3 is a humanized anti-

CD3 IgG (Protein Design Lab); 5G1.1 is a humanized anti-complement factor 5 (C5) antibody (Alexion Pharm); D2E7 is a humanized anti-TNF-alpha antibody (CAT/BASF); CDP870 is a humanized anti-TNF-alpha Fab fragment (Celltech); IDEC-151 is a primatized anti-CD4 IgG1 antibody (IDEC Pharm/SmithKline Beecham); MDX-CD4 is a human anti-CD4 IgG antibody (Medarex/Eisai/Genmab); CD20-sreptdavidin (+biotin-yttrium 90; NeoRx); CDP571 is a humanized anti-TNF-alpha IgG4 antibody (Celltech); LDP-02 is a humanized anti- $\alpha_v\beta_7$antibody (LeukoSite/Genentech); OrthoClone OKT4A is a humanized anti-CD4 IgG antibody (Ortho Biotech); ANTOVA™ is a humanized anti-CD40L IgG antibody (Biogen); ANTEGREN™ is a humanized anti-VLA-4 IgG antibody (Elan); and CAT-152 is a human anti-TGF-beta$_2$ antibody (Cambridge Ab Tech).

[00289] In another specific embodiment, the methods of the invention encompass administration of a *Listeria*-based EphA2 vaccine of the invention in combination with the administration of one or more prophylactic/therapeutic agents that are angiogenesis inhibitors such as, but not limited to: Angiostatin (plasminogen fragment); antiangiogenic antithrombin III; Angiozyme; ABT-627; Bay 12-9566; Benefin; Bevacizumab (AVASTIN™); BMS-275291; cartilage-derived inhibitor (CDI); CAI; CD59 complement fragment; CEP-7055; Col 3; Combretastatin A-4; Endostatin (collagen XVIII fragment); fibronectin fragment; Gro-beta; Halofuginone; Heparinases; Heparin hexasaccharide fragment; HMV833; Human chorionic gonadotropin (hCG); IM-862; Interferon alpha/beta/gamma; Interferon inducible protein (IP-10); Interleukin-12; Kringle 5 (plasminogen fragment); Marimastat; Metalloproteinase inhibitors (TIMPs); 2-Methoxyestradiol; MMI 270 (CGS 27023A); MoAb IMC-1C11; Neovastat; NM-3; Panzem; PI-88; Placental ribonuclease inhibitor; Plasminogen activator inhibitor; Platelet factor-4 (PF4); Prinomastat; Prolactin 16kD fragment; Proliferin-related protein (PRP); PTK 787/ZK 222594; Retinoids; Solimastat; Squalamine; SS 3304; SU 5416; SU6668; SU11248; Tetrahydrocortisol-S; tetrathiomolybdate; thalidomide; Thrombospondin-1 (TSP-1); TNP-470; Transforming growth factor-beta (TGF-β); Vasculostatin; Vasostatin (calreticulin fragment); ZD6126; ZD6474; farnesyl transferase inhibitors (FTI); and bisphosphonates.

[00290] In another specific embodiment, the methods of the invention encompass administration of an EphA2 vaccine of the invention in combination with the administration of one or more prophylactic/therapeutic agents that are anti-cancer agents such as, but not limited to: acivicin, aclarubicin, acodazole hydrochloride, acronine, adozelesin, aldesleukin, altretamine, ambomycin, ametantrone acetate, aminoglutethimide, amsacrine, anastrozole, anthramycin, asparaginase, asperlin, azacitidine, azetepa, azotomycin,

batimastat, benzodepa, bicalutamide, bisantrene hydrochloride, bisnafide dimesylate, bizelesin, bleomycin sulfate, brequinar sodium, bropirimine, busulfan, cactinomycin, calusterone, caracemide, carbetimer, carboplatin, carmustine, carubicin hydrochloride, carzelesin, cedefingol, chlorambucil, cirolemycin, cisplatin, cladribine, crisnatol mesylate, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin hydrochloride, decarbazine, decitabine, dexormaplatin, dezaguanine, dezaguanine mesylate, diaziquone, docetaxel, doxorubicin, doxorubicin hydrochloride, droloxifene, droloxifene citrate, dromostanolone propionate, duazomycin, edatrexate, eflornithine hydrochloride, elsamitrucin, enloplatin, enpromate, soluble EphrinA1, EphrinA1-Fc polypeptides, EphA2-Fc polypeptides, EphA2 antisense, EphrinA1 antisense, epipropidine, epirubicin hydrochloride, erbulozole, esorubicin hydrochloride, estramustine, estramustine phosphate sodium, etanidazole, etoposide, etoposide phosphate, etoprine, fadrozole hydrochloride, fazarabine, fenretinide, floxuridine, fludarabine phosphate, fluorouracil, flurocitabine, fosquidone, fostriecin sodium, gemcitabine, gemcitabine hydrochloride, hydroxyurea, idarubicin hydrochloride, ifosfamide, ilmofosine, interleukin 2 (including recombinant interleukin 2, or rIL2), interferon alpha-2a, interferon alpha-2b, interferon alpha-n1, interferon alpha-n3, interferon beta-I a, interferon gamma-I b, iproplatin, irinotecan hydrochloride, lanreotide acetate, letrozole, leuprolide acetate, liarozole hydrochloride, lometrexol sodium, lomustine, losoxantrone hydrochloride, masoprocol, maytansine, mechlorethamine hydrochloride, megestrol acetate, melengestrol acetate, melphalan, menogaril, mercaptopurine, methotrexate, methotrexate sodium, metoprine, meturedepa, mitindomide, mitocarcin, mitocromin, mitogillin, mitomalcin, mitomycin, mitosper, mitotane, mitoxantrone hydrochloride, mycophenolic acid, nitrosoureas, nocodazole, nogalamycin, ormaplatin, oxisuran, paclitaxel, pegaspargase, peliomycin, pentamustine, peplomycin sulfate, perfosfamide, pipobroman, piposulfan, piroxantrone hydrochloride, plicamycin, plomestane, porfimer sodium, porfiromycin, prednimustine, procarbazine hydrochloride, puromycin, puromycin hydrochloride, pyrazofurin, riboprine, rogletimide, safingol, safingol hydrochloride, semustine, simtrazene, sparfosate sodium, sparsomycin, spirogermanium hydrochloride, spiromustine, spiroplatin, streptonigrin, streptozocin, sulofenur, talisomycin, tecogalan sodium, tegafur, teloxantrone hydrochloride, temoporfin, teniposide, teroxirone, testolactone, thiamiprine, thioguanine, thiotepa, tiazofurin, tirapazamine, toremifene citrate, trastuzumab (HERCEPTIN™), trestolone acetate, triciribine phosphate, trimetrexate, trimetrexate glucuronate, triptorelin, tubulozole hydrochloride, uracil mustard, uredepa, vapreotide, verteporfin, vinblastine sulfate, vincristine sulfate, vindesine, vindesine sulfate, vinepidine sulfate, vinglycinate sulfate,

vinleurosine sulfate, vinorelbine tartrate, vinrosidine sulfate, vinzolidine sulfate, vorozole, zeniplatin, zinostatin, zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3, 5-ethynyluracil, abiraterone, aclarubicin, acylfulvene, adecypenol, adozelesin, aldesleukin, ALL-TK antagonists, altretamine, ambamustine, amidox, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, andrographolide, angiogenesis inhibitors, antagonist D, antagonist G, antarelix, anti-dorsalizing morphogenetic protein-1, antiandrogens, antiestrogens, antineoplaston, aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ara-CDP-DL-PTBA, arginine deaminase, asulacrine, atamestane, atrimustine, axinastatin 1, axinastatin 2, axinastatin 3, azasetron, azatoxin, azatyrosine, baccatin III derivatives, balanol, batimastat, BCR/ABL antagonists, benzochlorins, benzoylstaurosporine, beta lactam derivatives, beta-alethine, betaclamycin B, betulinic acid, bFGF inhibitor, bicalutamide, bisantrene, bisaziridinylspermine, bisnafide, bistratene A, bizelesin, breflate, bropirimine, budotitane, buthionine sulfoximine, calcipotriol, calphostin C, camptothecin derivatives, canarypox IL-2, capecitabine, carboxamide-amino-triazole, carboxyamidotriazole, CaRest M3, CARN 700, cartilage derived inhibitor, carzelesin, casein kinase inhibitors (ICOS), castanospermine, cecropin B, cetrorelix, chloroquinoxaline sulfonamide, cicaprost, cis-porphyrin, cladribine, clomifene analogues, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analogue, conagenin, crambescidin 816, crisnatol, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentanthraquinones, cycloplatam, cypemycin, cytarabine ocfosfate, cytolytic factor, cytostatin, dacliximab, decitabine, dehydrodidemnin B, deslorelin, dexamethasone, dexifosfamide, dexrazoxane, dexverapamil, diaziquone, didemnin B, didox, diethylnorspermine, dihydro-5-azacytidine, dihydrotaxol, dioxamycin, diphenyl spiromustine, docetaxel, docosanol, dolasetron, doxifluridine, droloxifene, dronabinol, duocarmycin SA, ebselen, ecomustine, edelfosine, edrecolomab, eflornithine, elemene, emitefur, epirubicin, epristeride, estramustine analogue, estrogen agonists, estrogen antagonists, etanidazole, etoposide phosphate, exemestane, fadrozole, fazarabine, fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, fluasterone, fludarabine, fluorodaunorunicin hydrochloride, forfenimex, formestane, fostriecin, fotemustine, gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors, gemcitabine, glutathione inhibitors, hepsulfam, heregulin, hexamethylene bisacetamide, hypericin, ibandronic acid, idarubicin, idoxifene, idramantone, ilmofosine, ilomastat, imidazoacridones, imiquimod, immunostimulant peptides, insulin-like growth factor-1 receptor inhibitor, interferon agonists, interferons, interleukins, iobenguane,

iododoxorubicin, ipomeanol, iroplact, irsogladine, isobengazole, isohomohalicondrin B, itasetron, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, leinamycin, lenograstim, lentinan sulfate, leptolstatin, letrozole, leukemia inhibiting factor, leukocyte alpha interferon, leuprolide+estrogen+progesterone, leuprorelin, levamisole, liarozole, linear polyamine analogue, lipophilic disaccharide peptide, lipophilic platinum compounds, lissoclinamide 7, lobaplatin, lombricine, lometrexol, lonidamine, losoxantrone, lovastatin, loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, lytic peptides, maitansine, mannostatin A, marimastat, masoprocol, maspin, matrilysin inhibitors, matrix metalloproteinase inhibitors, menogaril, merbarone, meterelin, methioninase, metoclopramide, MIF inhibitor, mifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mitoguazone, mitolactol, mitomycin analogues, mitonafide, mitotoxin fibroblast growth factor-saporin, mitoxantrone, mofarotene, molgramostim, human chorionic gonadotrophin, monophosphoryl lipid A+myobacterium cell wall sk, mopidamol, multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy, mustard anticancer agent, mycaperoxide B, mycobacterial cell wall extract, myriaporone, N-acetyldinaline, N-substituted benzamides, nafarelin, nagrestip, naloxone+pentazocine, napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral endopeptidase, nilutamide, nisamycin, nitric oxide modulators, nitroxide antioxidant, nitrullyn, O6-benzylguanine, octreotide, okicenone, oligonucleotides, onapristone, ondansetron, ondansetron, oracin, oral cytokine inducer, ormaplatin, osaterone, oxaliplatin, oxaunomycin, paclitaxel, paclitaxel analogues, paclitaxel derivatives, palauamine, palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine, pegaspargase, peldesine, pentosan polysulfate sodium, pentostatin, pentrozole, perflubron, perfosfamide, perillyl alcohol, phenazinomycin, phenylacetate, phosphatase inhibitors, picibanil, pilocarpine hydrochloride, pirarubicin, piritrexim, placetin A, placetin B, plasminogen activator inhibitor, platinum complex, platinum compounds, platinum-triamine complex, porfimer sodium, porfiromycin, prednisone, propyl bis-acridone, prostaglandin J2, proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitor, protein kinase C inhibitors, microalgal, protein tyrosine phosphatase inhibitors, purine nucleoside phosphorylase inhibitors, purpurins, pyrazoloacridine, pyridoxylated hemoglobin polyoxyethylene conjugate, raf antagonists, raltitrexed, ramosetron, ras farnesyl protein transferase inhibitors, ras inhibitors, ras-GAP inhibitor, retelliptine demethylated, rhenium Re 186 etidronate, rhizoxin, ribozymes, RII retinamide, rogletimide, rohitukine, romurtide, roquinimex, rubiginone B1, ruboxyl, safingol, saintopin, SarCNU, sarcophytol A, sargramostim, Sdi 1 mimetics, semustine, senescence derived inhibitor 1, sense

oligonucleotides, signal transduction inhibitors, signal transduction modulators, single chain antigen binding protein, sizofiran, sobuzoxane, sodium borocaptate, sodium phenylacetate, solverol, somatomedin binding protein, sonermin, sparfosic acid, spicamycin D, spiromustine, splenopentin, spongistatin 1, squalamine, stem cell inhibitor, stem-cell division inhibitors, stipiamide, stromelysin inhibitors, sulfinosine, superactive vasoactive intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic glycosaminoglycans, tallimustine, tamoxifen methiodide, tauromustine, taxol, tazarotene, tecogalan sodium, tegafur, tellurapyrylium, telomerase inhibitors, temoporfin, temozolomide, teniposide, tetrachlorodecaoxide, tetrazomine, thaliblastine, thalidomide, thiocoraline, thioguanine, thrombopoietin, thrombopoietin mimetic, thymalfasin, thymopoietin receptor agonist, thymotrinan, thyroid stimulating hormone, tin ethyl etiopurpurin, tirapazamine, titanocene bichloride, topsentin, toremifene, totipotent stem cell factor, translation inhibitors, tretinoin, triacetyluridine, triciribine, trimetrexate, triptorelin, tropisetron, turosteride, tyrosine kinase inhibitors, tyrphostins, UBC inhibitors, ubenimex, urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists, vapreotide, variolin B, vector system, erythrocyte gene therapy, velaresol, veramine, verdins, verteporfin, vinorelbine, vinxaltine, vitaxin, vorozole, zanoterone, zeniplatin, zilascorb, and zinostatin stimalamer. Preferred additional anti-cancer drugs are 5-fluorouracil and leucovorin.

[00291] In more particular embodiments, the present invention also comprises the administration of one or more *Listeria*-based EphA2 vaccines of the invention in combination with the administration of one or more therapies such as, but not limited to anti-cancer agents such as those disclosed in Table 5 below, preferably for the treatment of breast, ovary, melanoma, prostate, colon and lung cancers as described above.

TABLE 5

Therapeutic Agent	Administration	Dose	Intervals
doxorubicin hydrochloride (Adriamycin RDF® and Adriamycin PFS®)	Intravenous	60-75 mg/m^2 on Day 1	21 day intervals
epirubicin hydrochloride (Ellence™)	Intravenous	100-120 mg/m^2 on Day 1 of each cycle or divided equally and given on Days 1-8 of the cycle	3-4 week cycles
fluorousacil	Intravenous	How supplied: 5 ml and 10 ml vials (containing 250 and 500 mg flourouracil respectively)	
docetaxel	Intravenous	60- 100 mg/m^2 over 1 hour	Once every 3 weeks

Therapeutic Agent	Administration	Dose	Intervals
(Taxotere®)			
paclitaxel (Taxol®)	Intravenous	175 mg/m^2 over 3 hours	Every 3 weeks for 4 courses (administered sequentially to doxorubicin-containing combination chemotherapy)
tamoxifen citrate (Nolvadex®)	Oral (tablet)	20-40 mg Dosages greater than 20 mg should be given in divided doses (morning and evening)	Daily
leucovorin calcium for injection	Intravenous or intramuscular injection	How supplied: 350 mg vial	Dosage is unclear from text. PDR 3610
luprolide acetate (Lupron®)	Single subcutaneous injection	1 mg (0.2 ml or 20 unit mark)	Once a day
flutamide (Eulexin®)	Oral (capsule)	250 mg (capsules contain 125 mg flutamide each)	3 times a day at 8 hour intervals (total daily dosage 750 mg)
nilutamide (Nilandron®)	Oral (tablet)	300 mg or 150 mg (tablets contain 50 or 150 mg nilutamide each)	300 mg once a day for 30 days followed by 150 mg once a day
bicalutamide (Casodex®)	Oral (tablet)	50 mg (tablets contain 50 mg bicalutamide each)	Once a day
progesterone	Injection	USP in sesame oil 50 mg/ml	
ketoconazole (Nizoral®)	Cream	2% cream applied once or twice daily depending on symptoms	
prednisone	Oral (tablet)	Initial dosage may vary from 5 mg to 60 mg per day depending on the specific disease entity being treated.	
estramustine phosphate sodium (Emcyt®)	Oral (capsule)	14 mg/ kg of body weight (*i.e.* one 140 mg capsule for each 10 kg or 22 lb of body weight)	Daily given in 3 or 4 divided doses
etoposide or VP-16	Intravenous	5 ml of 20 mg/ ml solution (100 mg)	
dacarbazine (DTIC-Dome®)	Intravenous	2-4.5 mg/knowing	Once a day for 10 days. May be repeated at 4 week intervals
polifeprosan 20 with carmustine implant (BCNU) (nitrosourea) (Gliadel®)	wafer placed in resection cavity	8 wafers, each containing 7.7 mg of carmustine, for a total of 61.6 mg, if size and shape of resection cavity allows	
cisplatin	Injection	How supplied: solution of 1 mg/ml in multi-dose vials of 50mL and 100mL	
mitomycin	Injection	supplied in 5 mg and 20 mg vials (containing 5 mg and 20	

Therapeutic Agent	Administration	Dose	Intervals
		mg mitomycin)	
gemcitabine HCl (Gemzar®)	Intravenous	For NSCLC- 2 schedules have been investigated and the optimum schedule has not been determined 4 week schedule- administration intravenously at 1000 mg/m^2 over 30 minutes on 3 week schedule- Gemzar administered intravenously at 1250 mg/m^2 over 30 minutes	4 week schedule- Days 1,8 and 15 of each 28-day cycle. Cisplatin intravenously at 100 mg/m^2 on day 1 after the infusion of Gemzar. 3 week schedule- Days 1 and 8 of each 21 day cycle. Cisplatin at dosage of 100 mg/m^2 administered intravenously after administration of Gemzar on day 1.
carboplatin (Paraplatin®)	Intravenous	Single agent therapy: 360 mg/m^2 I.V. on day 1 (infusion lasting 15 minutes or longer) Other dosage calculations: Combination therapy with cyclophosphamide, Dose adjustment recommendations, Formula dosing, etc.	Every 4 weeks
Ifosamide (Ifex®)	Intravenous	1.2 g/m^2 daily	5 consecutive days Repeat every 3 weeks or after recovery from hematologic toxicity
Topotecan hydrochloride (Hycamtin®)	Intravenous	1.5 mg/m^2 by intravenous infusion over 30 minutes daily	5 consecutive days, starting on day 1 of 21 day course

[00292] The invention also encompasses administration of the *Listeria*-based EphA2 vaccines of the invention in combination with radiation therapy comprising the use of x-rays, gamma rays and other sources of radiation to destroy the cancer cells. In preferred embodiments, the radiation treatment is administered as external beam radiation or teletherapy wherein the radiation is directed from a remote source. In other preferred embodiments, the radiation treatment is administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass.

[00293] In a specific embodiment, the methods of the invention encompass administration of a *Listeria*-based EphA2 vaccine of the invention in combination with the administration of one or more anti-inflammatory agents. Any anti-inflammatory agent, including agents useful in therapies for inflammatory disorders, well-known to one of skill in the art can be used in the compositions and methods of the invention. Non-limiting examples of anti-inflammatory agents include non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs, anticholinergics (*e.g.*, atropine sulfate, atropine methylnitrate, and ipratropium bromide (ATROVENT™)), beta2-agonists (*e.g.*,

abuterol (VENTOLIN™ and PROVENTIL™), bitolterol (TORNALATE™), levalbuterol (XOPONEX™), metaproterenol (ALUPENT™), pirbuterol (MAXAIR™), terbutlaine (BRETHAIRE™ and BRETHINE™), albuterol (PROVENTIL™, REPETABS™, and VOLMAX™), formoterol (FORADIL AEROLIZER™), and salmeterol (SEREVENT™ and SEREVENT DISKUS™)), and methylxanthines (*e.g.*, theophylline (UNIPHYL™, THEO-DUR™, SLO-BID™, AND TEHO-42™)). Examples of NSAIDs include, but are not limited to, aspirin, ibuprofen, celecoxib (CELEBREX™), diclofenac (VOLTAREN™), etodolac (LODINE™), fenoprofen (NALFON™), indomethacin (INDOCIN™), ketoralac (TORADOL™), oxaprozin (DAYPRO™), nabumentone (RELAFEN™), sulindac (CLINORIL™), tolmentin (TOLECTIN™), rofecoxib (VIOXX™), naproxen (ALEVE™, NAPROSYN™), ketoprofen (ACTRON™) and nabumetone (RELAFEN™). Such NSAIDs function by inhibiting a cyclooxgenase enzyme (*e.g.*, COX-1 and/or COX-2). Examples of steroidal anti-inflammatory drugs include, but are not limited to, glucocorticoids, dexamethasone (DECADRON™), corticosteroids (*e.g.*, methylprednisolone (MEDROL™)), cortisone, hydrocortisone, prednisone (PREDNISONE™ and DELTASONE™), prednisolone (PRELONE™ and PEDIAPRED™), triamcinolone, azulfidine, and inhibitors of eicosanoids (*e.g.*, prostaglandins, thromboxanes, and leukotrienes (*see* Table 6, *infra*, for non-limiting examples of leukotriene and typical dosages of such agents)).

[00294] In certain embodiments, the anti-inflammatory agent is an agent useful in the prevention, management, treatment, and/or amelioration of asthma or one or more symptoms thereof. Non-limiting examples of such agents include adrenergic stimulants (*e.g.*, catecholamines (*e.g.*, epinephrine, isoproterenol, and isoetharine), resorcinols (*e.g.*, metaproterenol, terbutaline, and fenoterol), and saligenins (*e.g.*, salbutamol)), adrenocorticoids, blucocorticoids, corticosteroids (*e.g.*, beclomethadonse, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, and prednisone), other steroids, beta2-agonists (*e.g.*, albtuerol, bitolterol, fenoterol, isoetharine, metaproterenol, pirbuterol, salbutamol, terbutaline, formoterol, salmeterol, and albutamol terbutaline), anti-cholinergics (*e.g.*, ipratropium bromide and oxitropium bromide), IL-4 antagonists (including antibodies), IL-5 antagonists (including antibodies), IL-13 antagonists (including antibodies), PDE4-inhibitor, NF-Kappa-β inhibitor, VLA-4 inhibitor, CpG, anti-CD23, selectin antagonists (TBC 1269), mast cell protease inhibitors (*e.g.*, tryptase kinase inhibitors (*e.g.*, GW-45, GW-58, and genisteine), phosphatidylinositide-3' (PI3)-kinase inhibitors (*e.g.*, calphostin C), and other kinase inhibitors (*e.g.*, staurosporine) (see Temkin *et al.*, 2002 J Immunol 169(5):2662-2669; Vosseller *et al.*, 1997 Mol. Biol.

Cell 8(5):909-922; and Nagai *et al.*, 1995 Biochem Biophys Res Commun 208(2):576-581)), a C3 receptor antagonists (including antibodies), immunosuppressant agents (*e.g.*, methotrexate and gold salts), mast cell modulators (*e.g.*, cromolyn sodium (INTAL™) and nedocromil sodium (TILADE™)), and mucolytic agents (*e.g.*, acetylcysteine)). In a specific embodiment, the anti-inflammatory agent is a leukotriene inhibitor (*e.g.*, montelukast (SINGULAIR™), zafirlukast (ACCOLATE™), pranlukast (ONON™), or zileuton (ZYFLO™) (*see* Table 6)).

Table 6. Leukotriene Inhibitors for Asthma Therapy

Leukotriene Modifier	Usual Daily Dosage
Montelukast (SINGULAIR™)	4 mg for 2-5 years old 5 mg for 6 to 15 years old 10mg for 15 years and older
Zafirlukast (ACCOLATE™)	10 mg b.i.d. for 5 to 12 years old twice daily 20 mg b.i.d. for 12 years or older twice daily
Pranlukast (ONON™)	Only avialable in Asia
Zyleuton (ZYFLO™)	600 mg four times a day for 12 years and older

Table 7. H_1 Antihistamines

Chemical class and representative drugs	**Usual daily dosage**
Ethanolamine Diphehydramine Clemastine	 25-50 mg every 4-6 hours 0.34-2.68 mg every 12 hours
Ethylenediamine Tripelennamine	 25-50 mg every 4-6 hours
Alkylamine Brompheniramine Chlorpheniramine Triprolidine (1.25 mg/5ml)	 4 mg every 4-6 hours; or 8-12 mg of SR form every 8-12 hour 4 mg every 4-6 hours; or 8-12 mg of SR form every 8-12 hour 2.5 mg every 4-6 hours
Phenothiazine Promethazine	 25 mg at bedtime
Piperazine Hydroxyzine	 25 mg every 6-8 hours
Piperidines Astemizole (nonsedating) Azatadine Cetirzine Cyproheptadine Fexofenadine (nonsedating) Loratidine (nonsedating)	 10 mg/day 1-2 mg every 12 hours 10 mg/day 4 mg every 6-8 hour 60 mg every 12 hours 10 mg every 24 hours

[00295] Cancer therapies as well as therapies for hyperproliferative cell disorders other than cancer and their dosages, routes of administration and recommended usage are

known in the art and have been described in such literature as the *Physician's Desk Reference* (56th ed., 2002, 57th ed., 2003, and 58th ed., 2004).

[00296]

5.5. Biological Activity

[00297] Toxicity and efficacy of the prophylactic and/or therapeutic protocols of the instant invention can be determined by standard pharmaceutical procedures in experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Prophylactic and/or therapeutic agents that exhibit large therapeutic indices are preferred. While prophylactic and/or therapeutic agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00298] The data obtained from the animal studies can be used in formulating a range of dosage of the prophylactic and/or therapeutic agents for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the vaccine or test compound that achieves a half-maximal inhibition of symptoms) as determined in animal studies. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00299] The anti-cancer activity of the therapies used in accordance with the present invention also can be determined by using various experimental animal models for the study of cancer, such as an immunocompetent mouse model, *e.g.*, Balb/c or C57/Bl/6, or transgenic mice where a mouse EphA2 is replaced with the human EphA2, mouse models to which murine tumor cell lines engineered to express human EphA2 are administered, animal models described in Section 6 *infra*, or any animal model (including hamsters, rabbits, etc.) known in the art and described in *Relevance of Tumor Models for Anticancer Drug Development* (1999, eds. Fiebig and Burger); *Contributions to Oncology* (1999, Karger); *The Nude Mouse in Oncology Research* (1991, eds. Boven and Winograd); and

Anticancer Drug Development Guide (1997 ed. Teicher), herein incorporated by reference in their entireties.

[00300] Compounds for use in therapy can be tested in other suitable animal model systems prior to testing in humans, including but not limited to in rats, mice, chicken, cows, monkeys, rabbits, hamsters, *etc.*, for example, the animal models described above. The compounds can then be used in the appropriate clinical trials.

[00301] Further, any assays known to those skilled in the art can be used to evaluate the prophylactic and/or therapeutic utility of the combinatorial therapies disclosed herein for treatment or prevention of cancer.

5.6. <u>Vaccine Compositions</u>

[00302] The compositions of the invention include bulk drug compositions useful in the manufacture of non-pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of a prophylactic and/or therapeutic agent disclosed herein or a combination of those agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of one or more *Listeria*-based EphA2 vaccines of the invention. The *Listeria*-based EphA2 vaccines of the invention may comprise one or more EphA2 antigenic peptide-expressing *Listeria* and a pharmaceutically acceptable carrier.

[00303] In a specific embodiment, a composition of the invention comprises a *Listeria*-based EphA2 vaccine and an additional prophylactic or therapeutic, *e.g.*, anti-cancer, agent. In accordance with this embodiment, the composition may further comprise a pharmaceutically acceptable carrier.

[00304] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete) or, more preferably, MF59C.1 adjuvant available from Chiron, Emeryville, CA), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenouslv Saline solutions and aqueous dextrose and

glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00305] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00306] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

[00307] Various delivery systems are known and can be used to administer a *Listeria*-based EphA2 vaccine of the invention or the combination of a *Listeria*-based EphA2 vaccine of the invention and a prophylactic agent or therapeutic agent useful for preventing or treating cancer, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the EphA2 antigenic peptide, receptor-mediated endocytosis (*see, e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.* Methods of administering a *Listeria*-based EphA2 vaccine or the combination of a *Listeria*-based EphA2 vaccine of the invention and prophylactic or therapeutic agent, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal, inhaled, and oral routes). In a specific embodiment, a *Listeria*-based EphA2 vaccine of the invention or the combination of a *Listeria*-based EphA2 vaccine of the invention and prophylactic or therapeutic agent are administered intramuscularly, intravenously, or subcutaneously. The *Listeria*-based

EphA2 vaccine of the invention or the combination of a *Listeria*-based EphA2 vaccine of the invention and prophylactic or therapeutic agent may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local.

[00308] In a specific embodiment, it may be desirable to administer the *Listeria*-based EphA2 vaccine of the invention or the combination of a *Listeria*-based EphA2 vaccine of the invention and prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

[00309] In yet another embodiment, the *Listeria*-based EphA2 vaccine of the invention or the combination of a *Listeria*-based EphA2 vaccine of the invention and prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:20; Buchwald *et al.*, 1980, *Surgery* 88:507; Saudek *et al.*, 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the EphA2 antigenic peptide-expressing *Listeria* of the invention (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, FL (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; *see also* Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, *Ann. Neurol.* 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 7 1:105); U.S. Patent Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; 5,128,326; International Publication Nos. WO 99/15154 and WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be

placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

[00310] Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. *See, e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.*, 1996, *Radiotherapy & Oncology* 39:179-189; Song *et al.*, 1995, *PDA Journal of Pharmaceutical Science & Technology* 50:372-397; Cleek *et al.*, 1997, *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam *et al.*, 1997, *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760, each of which is incorporated herein by reference in its entirety.

5.6.1. <u>Formulations</u>

[00311] Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

[00312] Thus, the EphA2 antigenic peptide-expressing *Listeria* of the invention and their physiologically acceptable salts and solvates be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, parenteral or mucosal (such as buccal, vaginal, rectal, sublingual) administration. In a preferred embodiment, local or systemic parenteral administration is used.

[00313] For oral administration, the *Listeria*-based EphA2 vaccine may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or

propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[00314] Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

[00315] For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

[00316] For administration by inhalation, the prophylactic or therapeutic agents for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00317] The *Listeria*-based EphA2 vaccine may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[00318] The vaccines of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[00319] In addition to the formulations described previously, the prophylactic or therapeutic agents may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the prophylactic or therapeutic agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[00320] The invention also provides that a *Listeria*-based EphA2 vaccine of the invention is packaged in a hermetically sealed container such as an ampoule or sachette

indicating the quantity. In one embodiment, the vaccine is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject.

[00321] In a preferred embodiment of the invention, the formulation and administration of various chemotherapeutic, biological/immunotherapeutic and hormonal therapeutic agents for use in combination with the vaccine of the invention are known in the art and often described in the *Physician's Desk Reference,* (56th ed. 2002). For instance, in certain specific embodiments of the invention, the agents can be formulated and supplied as provided in Table 3.

[00322] In other embodiments of the invention, radiation therapy agents such as radioactive isotopes can be given orally as liquids in capsules or as a drink. Radioactive isotopes can also be formulated for intravenous injections. The skilled oncologist can determine the preferred formulation and route of administration.

[00323] In certain embodiments, the EphA2 antigenic peptide-expressing *Listeria* of the invention are formulated at 1 mg/ml, 5 mg/ml, 10 mg/ml, and 25 mg/ml for intravenous injections and at 5 mg/ml, 10 mg/ml, and 80 mg/ml for repeated subcutaneous administration and intramuscular injection. In other embodiments, the EphA2 antigenic peptide-expressing *Listeria* of the invention are formulated at amounts ranging between approximately $1x10^2$ CFU/ml to approximately $1x10^{12}$ CFU/ml, for example at $1x10^2$ CFU/ml, $5x10^2$ CFU/ml, $1x10^3$ CFU/ml, $5x10^3$ CFU/ml, $1x10^4$ CFU/ml, $5x10^4$ CFU/ml, $1x10^5$ CFU/ml, $5x10^5$ CFU/ml, $1x10^6$ CFU/ml, $5x10^6$ CFU/ml, $1x10^7$ CFU/ml, $5x10^7$ CFU/ml, $1x10^8$ CFU/ml, $5x10^8$ CFU/ml, $1x10^9$ CFU/ml, $5x10^9$ CFU/ml, $1x10^{10}$ CFU/ml, $5x10^{10}$ CFU/ml, $1x10^{11}$ CFU/ml, $5x10^{11}$ CFU/ml, or $1x10^{12}$ CFU/ml.

[00324] The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

5.6.2. Dosages

[00325] The amount of the composition of the invention which will be effective in the treatment, prevention or management of cancer can be determined by standard research techniques. For example, the dosage of the *Listeria*-based EphA2 vaccine of the invention which will be effective in the treatment, prevention or management of cancer can be determined by administering the composition to an animal model such as, *e.g.*, the animal

models disclosed herein or known to those skilled in the art. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges.

[00326] Selection of the preferred effective dose can be determined (*e.g.*, via clinical trials) by a skilled artisan based upon the consideration of several factors which will be known to one of ordinary skill in the art. Such factors include the disease to be treated or prevented, the symptoms involved, the patient's body mass, the patient's immune status and other factors known by the skilled artisan to reflect the accuracy of administered pharmaceutical compositions.

[00327] The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the cancer, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[00328] With respect to the dosage of *Listeria* in the *Listeria*-based EphA2 vaccines of the invention, the dosage is based on the amount colony forming units (c.f.u.). Generally, in various embodiments, the dosage ranges are from about 1.0 c.f.u./kg to about 1×10^{10} c.f.u./kg; from about 1.0 c.f.u./kg to about 1×10^{8} c.f.u./kg; from about 1×10^{2} c.f.u./kg to about 1×10^{8} c.f.u./kg; and from about 1×10^{4} c.f.u./kg to about 1×10^{8} c.f.u./kg. Effective doses may be extrapolated from dose-response curves derived animal model test systems. In certain exemplary embodiments, the dosage ranges are 0.001-fold to 10,000-fold of the murine LD_{50}, 0.01-fold to 1,000-fold of the murine LD_{50}, 0.1-fold to 500-fold of the murine LD_{50}, 0.5-fold to 250-fold of the murine LD_{50}, 1-fold to 100-fold of the murine LD_{50}, and 5-fold to 50-fold of the murine LD_{50}. In certain specific embodiments, the dosage ranges are 0.00.1-fold, 0.01-fold, 0.1-fold, 0.5-fold, 1-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold, 500-fold, 1,000-fold, 5,000-fold or 10,000-fold of the murine LD_{50}.

[00329] For other cancer therapeutic agents administered to a patient, the typical doses of various cancer therapeutics known in the art are provided in Table 3. Given the invention, certain preferred embodiments will encompass the administration of lower dosages in combination treatment regimens than dosages recommended for the administration of single agents.

[00330] The invention provides for any method of administrating lower doses of known prophylactic or therapeutic agents than previously thought to be effective for the prevention, treatment, management or amelioration of cancer. Preferably, lower doses of

known anti-cancer therapies are administered in combination with lower doses of *Listeria*-based EphA2 vaccines of the invention.

5.7. <u>Kits</u>

[00331] The invention provides a pack or kit comprising one or more containers filled with a *Listeria*-based EphA2 vaccine of the invention or a component of a *Listeria*-based EphA2 vaccine of the invention. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a cancer or other hyperproliferative disorder can also be included in the pack or kit. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00332] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises one or more a *Listeria*-based EphA2 vaccines of the invention. In another embodiment, a kit further comprises one or more other prophylactic or therapeutic agents useful for the treatment of cancer or another hyperproliferative disorder, in one or more containers. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

6. EXAMPLES: *LISTERIA*-BASED EphA2 VACCINES PROVIDE THERAPEUTIC AND PROPHYLACTIC BENEFITS AGAINST EphA2-EXPRESSING CANCERS

[00333] The receptor tyrosine kinase EphA2 is selectively over-expressed in a variety of malignant cell types and tumors. Additionally, recent studies have identified patient-derived T lymphocytes that recognize EphA2. As such, EphA2 provides a much-needed target for active immunotherapy. Here, we show that ectopic expression of human EphA2 in the Gram-positive facultative intracellular bacterium *Listeria monocytogenes* (*Listeria*) can provide antigen-specific anti-tumor responses in vaccinated animals. *Listeria* infects critical antigen presenting cells and thereby provides efficacy as a cancer therapy based its ability to induce potent and robust CD4+ and CD8+ T cell responses against encoded antigens. Attenuated *Listeria* mutant strains, which retain the antigen delivery potency of wild-type bacteria, yet are nearly 10,000-fold less pathogenic in mice, were employed. To demonstrate the efficacy of a Listeria-based EphA2 vaccine, *Listeria* actA$^-$ strains were engineered to express the extracellular (ECD) or intracellular (ICD) domain of human EphA2 (actA-hEphA2-ECD or actA-hEphA2-ICD). Expression and secretion of hEphA2-EX and -CO from *Listeria* was confirmed by Western blot analysis. Protective

immunization with actA-hEphA2EX significantly inhibited the subcutaneous growth of CT26 cells that express full-length hEphA2 (p=0.0037). As controls, mice vaccinated with the parental actA strain developed tumors that were comparable to vehicle-treated control mice. Protective immunization with actA-hEphA2CO significantly increased the survival rate in mice challenged with RenCA-hEphA2. Subsequently, the therapeutic efficacy of actA-hEphA2-ECD or actA-hEphA2-ICD was evaluated using the experimental CT26-hEphA2 lung tumor model. Following intravenous implantation of tumor cells, Balb/c mice were immunized with actA, actA-hEphA2EX or actA-hEphA2-ICD. Immunization with either actA-hEphA2-ECD or actA-hEphA2-ICD significantly prolonged survival (median survival >43 days, p= 0.0035), as compared to matched controls (vehicle or actA median survival time was 19 and 20 days, respectively). Importantly, 80% of the huEphA2 immunized mice survived until Day 43 following tumor implantation. Together, these data demonstrate that Listeria-mediated vaccination targeting the EphA2 tumor antigen can provide both preventative and therapeutic efficacy against a variety of malignancies.

6.1. EXAMPLE 1: *LISTERIA* LIFE CYCLE

[00334] The life cycle of *Listeria monocytogenes*, encompassing the steps of endocytosis, phagolysosomal lysis, and cell to cell spread, are shown in Figure 1A-1B.

6.2. EXAMPLE 2: CONSTRUCTION OF EphA2-EXPRESSING AND CONTROL *LISTERIA* STRAINS

6.2.1. Background

[00335] Given the mechanisms by which *Listeria* programs the presentation of heterologous antigens via the MHC class I pathway, the efficiency of both expression of heterologous genes and secretion of the newly synthesized protein from the bacterium into the cytoplasm of the infected (antigen presenting) cell is related directly to the potency of CD8+ T cell priming and/or activation. As the level of Ag-specific T cell priming is related directly to vaccine efficacy, the efficiency of heterologous protein expression and secretion is linked directly to vaccine potency. Thus, the efficiency of EphA2 expression and secretion was optimized to maximize the potency of *Listeria*-based vaccines, in terms of priming and/or activating CD8+ T cell responses specific for the encoded EphA2 protein.

6.2.2. Preparation of mutant *Listeria* strains.

[00336] *Listeria* strains were derived from 10403S (Bishop *et al.*, *J. Immunol.* 139:2005 (1987)). *Listeria* strains with in-frame deletions of the indicated genes were generated by SOE-PCR and allelic exchange with established methods (Camilli *et al.*, *Mol.*

Microbiol. 8:143 (1993)). The mutant strain LLO L461T (DP-L4017) was described in Glomski, *et al.*, *J. Cell. Biol.* 156: 1029 (2002), incorporated by reference herein. The *actA*⁻ mutant (DP-L4029) is the DP-L3078 strain described in Skoble et al., *J. of Cell Biology*, 150: 527-537 (2000), incorporated by reference herein in its entirety, which has been cured of its prophage. (Prophage curing is described in (Lauer *et al.*, *J. Bacteriol.* 184:4177 (2002)); U.S. Patent Publication No. 2003/0203472.)

[00337] In some vaccines, mutant strains of Listeria that are deficient with respect to internalin B (Genbank accession number AL591975 (*Listeria monocytogenes* strain EGD, complete genome, segment 3/12; inlB gene region: nts. 97008-98963), incorporated by reference herein in its entirety, and/or the sequence listed as Genbank accession number NC_003210 (*Listeria monocytogenes* strain EGD, complete genome, *inlB* gene region: nts. 457008-458963), incorporated by reference herein in its entirety) are used. One particular *actA*⁻*inlB*⁻ strain (DP-L4029*inlB*) was deposited with the American Type Culture Collection (ATCC) on October 3, 2003, and designated with accession number PTA-5562).

6.2.3. Cloning vectors

[00338] Selected heterologous antigen expression cassette molecular constructs were inserted into pPL2 (Lauer et. al. J. Bacteriol. 2002), or pAM401 (Wirth et. al., J. Bacteriol. 165:831-836), modified to contain the multiple cloning sequence of pPL2 (Aat II small fragment, 171 bps), inserted between blunted *Xba I* and *Nru I* recognition sites, within the tetracycline resistance gene (pAM401-MCS). In general, the hly promoter and (selected) signal peptide sequence was inserted between the unique *Kpn I* and *Bam HI* sites in the pPL2 or pAM401-MCS plasmid vectors. Selected EphA2 genes (sometimes modified to contain N-terminal and C-terminal epitope tags; see description below) were cloned subsequently into these constructs between unique Bam HI and Sac I sites. Molecular constructs based on the pAM401-MCS plasmid vector were introduced by electroporation into selected *Listeria* monocytogenes strains also treated with lysozyme, utilizing methods common to those skilled in the art. The expected plasmid structure in *Listeria*-transfectants was verified by isolating DNA from colonies that formed on chloramphenicol-containing BHI agar plates (10 μg/ml) by restriction enzyme analysis. Recombinant *Listeria* transformed with various pAM401-MCS based heterologous protein expression cassette constructs were utilized to measure heterologous protein expression and secretion, as described below.

[00339] The pPL2 based heterologous protein expression cassette constructs were incorporated into the tRNAArg gene in the genome of selected *Listeria* strains, according to the methods as described previously (Lauer *et al.*, 2002, *J. Bacteriol.* 184:4177-4186).

Briefly, the pPL2 heterologous protein expression cassette constructs plasmid was first introduced into the E. coli host strain SM10 (Simon *et al.*, 1983, *Bio/Technology* 1:784-791) by electroporation or by chemical means. Subsequently, the pPL2-based plasmid was transferred from transformed SM10 to the selected *Listeria* strains by conjugation. Following incubation on drug-selective BHI agar plates containing 7.5 μg of chloramphenicol per ml and 200 μg of streptomycin per ml as described, selected colonies are purified by passaging 3 times on plates with the same composition. To verify integration of the pPL2 vector at the phage attachment site, individual colonies are picked and screened by PCR using the primer pair of forward primer NC16 (5'-gtcaaaacatacgctcttatc-3') (SEQ ID NO:47) and reverse primer PL95 (5'-acataatcagtccaaagtagatgc-3') (SEQ ID NO:48). Selected colonies having the pPL2-based plasmid incorporated into the tRNAArg gene in the genome of selected *Listeria* strains yielded a diagnostic DNA amplicon of 499 bps.

6.2.4. Promoter

[00340] Heterologous protein expression cassettes contained the prfA-dependent hly promoter, which drives the transcription of the gene encoding Listeriolysin O (LLO), and is activated within the microenvironment of the infected cell. Nucleotides 205586-206000 (414 bps) were amplified by PCR from *Listeria* monocytogenes, strain DP-L4056, using the primer pair shown below. The region amplified includes the hly promoter and also the first 28 amino acids of LLO, comprising the secA1 signal peptide (ibid) and PEST domain. The expected sequence of this region for *Listeria* monocytogenes, strain EGD can be found in GenBank (Accession number: gi|16802048|ref|NC_003210.1|[16802048]).

[00341] *Primer Pair*

[00342] *Forward* (KpnI-LLO nts. 1257-1276):

[00343] 5'-CTCT<u>GGTACC</u>TCCTTTGATTAGTATATTC (SEQ ID NO:49)

[00344] *Reverse* (Bam HI-LLO nts. X-x):

[00345] 5'-CTCT<u>GGATCC</u>ATCCGCGTGTTTCTTTTCG (SEQ ID NO:50)

[00346] (Restriction endonuclease recognition sites are underlined)

[00347] The 422 bp PCR amplicon was cloned into the plasmid vector pCR-XL-TOPO (Invitrogen, Carlsbad, CA), according to the manufacturer's specifications. The nucleotide sequences of *Listeria*-specific bases in the pCR-XL-TOPO-hly promoter plasmid clone was determined. *Listeria* monocytogenes strain DP-L4056 contained eight nucleotide base changes flanking the prfA box in the hly promoter, as compared to the EGD strain. The hly promoter alignment for the *Listeria* monocytogenes DP-L4056 and EGD strains is shown in the Figure below (SEQ ID NOs: 68 and 69, respectively).

Listeria hly DP-L4056 and EGD Alignment

```
Query:       Listeria EGD
Subject:     DP-L4056 (wild-type, Portnoy strain)

                                                                   prfA Box
Query: 1     ggtacctcctttgattagtatattcctatcttaaagtgacttttatgttgaggcattaac 60
             |||||||||||||||||||||||||||||||||||| |||||||||| ||||||||||
Sbjct: 1     ggtacctcctttgattagtatattcctatcttaaagttacttttatgtggaggcattaac 60

Query: 61    atttgttaacgacgataaagggacagcaggactagaataaagctataaagcaagcatata 120
             |||||||| ||||  ||| ||| |||| ||||||||||||||||||||||||||||||
Sbjct: 61    atttgttaatgacgtcaaaaggatagcaagactagaataaagctataaagcaagcatata 120

Query: 121   atattgcgtttcatctttagaagcgaatttcgccaatattataattatcaaaagagaggg 180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 121   atattgcgtttcatctttagaagcgaatttcgccaatattataattatcaaaagagaggg 180

                                               Shine-Delgarno    LLO start
Query: 181   gtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatg 240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 181   gtggcaaacggtatttggcattattaggttaaaaaatgtagaaggagagtgaaacccatg 240
```

[00348] The 422 bp DNA corresponding to the hly promoter and secA1 LLO signal peptide were liberated from the pCR-XL-TOPO-hly promoter plasmid clone by digestion with *Kpn I* and *Bam HI*, and cloned into the pPL2 plasmid vector (Lauer *et al.*, 2002, *J. Bact.*), according to conventional methods well-known to those skilled in the art. This plasmid is known as pPL2-hlyP (native).

6.2.5. Cloning and Insertion of EphA2 into pPL2 vectors for expression in selected recombinant *Listeria monocytogenes* strains

[00349] The external (EX2) and cytoplasmic (CO) domains of EphA2 which flank the EphA2 transmembrane helix were cloned separately for insertion into various pPL2-signal peptide expression constructs. Genes corresponding to the native mammalian sequence or codon-optimized for expression in *Listeria monocytogenes* of EphA2 EX2 and CO domains were used. The optimal codons in *Listeria* (see table, *ibid*) for each of the 20 amino acids were utilized for codon-optimized EphA2 EX2 and EphA2 CO. The codon-optimized EphA2 EX2 and CO domains were synthesized by extension of overlapping oligonucleotides, using techniques common to those skilled in the art. The expected sequence of all synthesized EphA2 constructs was verified by nucleotide sequencing.

[00350] SEQ ID NOS:23, 21 and 22 represent the primary amino acid sequences, together with the native and codon-optimized nucleotide sequences, respectively, for the EX2 domain of EphA2.

[00351] SEQ ID NOS: 34, 32 and 33 represent the primary amino acid sequences, together with the native and codon-optimized nucleotide sequences, respectivley, for the CO domain of EphA2.

[00352] Additonally, FLAG (Stratagene, La Jolla, CA) and myc epitope tags were inserted, respectively, in-frame at the amino and carboxy termini of synthesized EphA2

EX2 and CO genes for detection of expressed and secreted EphA2 by Western blot analysis using antibodies specific for the FLAG or proteins. Thus, the expressed protein had the following ordered elements: NH_2-Signal Peptide-FLAG-EphA2-myc-CO_2. Shown below are the FLAG and myc epitope tag amino acid and codon-optimized nucleotide sequences.

[00353] FLAG

[00354] 5'-GATTATAAAGATGATGATGATAAA (SEQ ID NO:51)

[00355] NH_2-DYKDDDDK-CO_2 (SEQ ID NO:52)

[00356] Myc

[00357] 5'-GAACAAAAATTAATTAGTGAAGAAGATTTA (SEQ ID NO:53)

[00358] NH_2-EQKLISEEDL-CO_2 (SEQ ID NO:54)

6.2.6. Detection of synthesized and secreted heterologous proteins by Western blot analysis

[00359] Synthesis of EphA2 protein and secretion from various selected recombinant *Listeria*-EphA2 strains was determined by Western blot analysis of trichloroacetic acid (TCA) precipitated bacterial culture fluids. Briefly, mid-log phase cultures of *Listeria* grown in BHI media were collected in a 50 mL conical centrifuge tube, the bacteria were pelleted, and ice-cold TCA was added to a final [6%] concentration to the bacterial culture supernatant and incubated on ice minimally for 90 min or overnight. The TCA-precipitated proteins were collected by centrifugation at 2400 X g for 20 min at 4°C. The pellet was then resuspended in 300-600 μl volume of TE, pH 8.0 containing 15 μg/ml phenol red. Sample dissolution was facilitated by vortexing. Sample pH was adjusted by NH_4OH addition if necessary until color was pink. All samples were prepared for electrophoresis by addition of 100 μl of 4X SDS loading buffer and incubating for 10 min. at 90°C. The samples were then centrifuged from 5 min at 14,000 rpm in a micro-centrifuge, and the supernatants collected and stored at –20°C. For Western bolt analysis, 20 μl of prepared fractions (the equivalent of culture fluids from of 1-4 x 10^9 bacteria), were loaded on the 4-12% SDS-PAGE gel, electrophoresed, and the proteins were transferred to PDDF membrane, according to common methods used by those skilled in the art. Transferred membranes were prepared s for incubation with antibody, by incubating in 5% dry milk in PBS for 2 hr. at room temperature with agitation. Antibodies were used under the following dilutions in PBST buffer (0.1% Tween 20 in PBS): (1) Rabbit anti-Myc polyclonal antibody (ICL laboratories, Newberg, Oregon) at 1:10,000; (2) murine anti-FLAG monoclonal antibody (Stratagene, *ibid*) at 1:2,000; and, (3) Rabbit anti-EphA2 (carboxy terminus-specific) polyclonal antibody (sc-924, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Specific binding of antibody to protein targets was evaluated by

secondary incubation with goat anti-rabbit or anti-mouse antibody conjugated with horseradish peroxidase and detection with the ECL chemilumenescence assay kit (Amersham), and exposure to film.

6.2.7. Secretion of EphA2 protein by recombinant *Listeria* encoding various forms of EphA2

6.2.7.1. *Listeria*: [strains DP-L4029 (*actA*) or DP-L4017 (LLO L461T)]

[00360] Expression cassette construct: LLOss-PEST-CO-EphA2 (SEQ ID NO:35)

[00361] The native sequence of the EphA2 CO domain was genetically fused to the native secA1 LLO sequence, and the heterologous antigen expression cassette under control of the *Listeria hly* promoter was inserted into the pPL2 plasmid between the *Kpn I* and *Sac I* sites as described (*ibid*). The pPL2-EphA2 plasmid constructs were introduced by conjugation into the *Listeria* strains DP-L4029 (actA) and DP-L4017 (L461T LLO) as described (*ibid*). Figure 2 shows the results of a Western blot analysis of TCA-precipitated bacterial culture fluids of 4029-EphA2 CO and 4017-EphA2 CO. This analysis demonstrated that recombinant *Listeria* engineered to contain a heterologous protein expression cassette comprised of native sequences corresponding to the secA1 and EphA2 CO fusion protein secreted multiple EphA2-specific fragments that were lower than the 52 kDa expected molecular weight, demonstrating the need for modification of the expression cassette.

6.2.7.2. *Listeria*: [DP-L4029 (*actA*)]

[00362] Expression cassette constructs:

Native LLOss-PEST-FLAG-EX2_EphA2-myc-CodonOp (SEQ ID NO:26)

(CodonOp) LLOss-PEST-(CodonOp)FLAG-EX2_EphA2-myc (SEQ ID NO:28)

[00363] The native secA1 LLO signal peptide sequence or secA1 LLO signal peptide sequence codon-optimized for expression in *Listeria* was fused genetically with the EphA2 EX2 domain sequence codon-optimized for expression in *Listeria*, and the heterologous antigen expression cassette under control of the *Listeria hly* promoter was inserted into the pPL2 plasmid between the *Kpn I* and *Sac I* sites as described (*ibid*). The pPL2-EphA2 plasmid constructs were introduced by conjugation into the *Listeria* strain DP-L4029 (actA) as described (*ibid*). Figure 3 shows the results of a Western blot analysis of TCA-precipitated bacterial culture fluids of *Listeria* actA encoding either the native or codon-optimized secA1 LLO signal peptide fused with the codon-optimized EphA2 EX2 domain.

This analysis demonstrated that the combination of utilizing sequence for both signal peptide and heterologous protein optimized for the preferred codon usage in *Listeria monocytogenes* resulted in expression of the expected full-length EphA2 EX2 domain protein. Expression of full-length EphA2 EX2 domain protein was poor with codon-optimization of the EphA2 coding sequence alone. The level of heterologous protein expression (fragmented or full-length) was highest when utilizing the *Listeria monocytogenes* LLO secA1 signal peptide, codon-optimized for expression in *Listeria monocytogenes*.

6.2.7.3. *Listeria*: [DP-L4029 (*actA*)]

[00364] Expression cassette constructs:

Native LLOss-PEST-(CodonOp) FLAG-EphA2_CO-myc (SEQ ID NO:37)

CodonOp LLOss-PEST-(CodonOp) FLAG- EphA2_CO-myc (SEQ ID NO:39)

CodonOp PhoD-(CodonOp) FLAG- EphA2_CO-myc (SEQ ID NO:41)

[00365] The native secA1 LLO signal peptide sequence or the secA1 LLO signal peptide sequence codon-optimized for expression in *Listeria*, or, alternatively, the Tat signal peptide of the phoD gene from *Bacillus subtilis* codon-optimized for expression in *Listeria*, was fused genetically with the EphA2 CO domain sequence codon-optimized for expression in *Listeria*, and the heterologous antigen expression cassette under control of the *Listeria hly* promoter was inserted into the pAM401-MCS plasmid between the *Kpn I* and *Sac I* sites as described (*ibid*). The pAM401-EphA2 plasmid constructs were introduced by electroporation into the *Listeria* strain DP-L4029 (actA) as described (*ibid*). Figure 4 shows the results of a Western blot analysis of TCA-precipitated bacterial culture fluids of *Listeria* actA encoding either the native or codon-optimized secA1 LLO signal peptide, or codon-optimized *Bacillus subtilis phoD* Tat signal peptide fused with the codon-optimized EphA2 CO domain. This analysis demonstrated once again that the combination of utilizing sequence for both signal peptide and heterologous protein optimized for the preferred codon usage in *Listeria monocytogenes* resulted in expression of the expected full-length EphA2 CO domain protein. Furthermore, expression and secretion of the expected full-length EphA2 CO domain protein resulted from recombinant *Listeria* encoding codon-optimized *Bacillus subtilis phoD* Tat signal peptide fused with the codon-optimized EphA2 CO domain. This result demonstrates the novel and unexpected finding that signal peptides from distinct bacterial species can be utilized to program the secretion of heterologous proteins from recombinant *Listeria*. Expression of full-length EphA2 CO domain protein

was poor with codon-optimization of just the EphA2 sequence. The level of heterologous protein expression was highest when utilizing signal peptides codon-optimized for expression in *Listeria monocytogenes*.

6.2.8. Construction of *Listeria* strains expressing AH1/OVA or AH1-A5/OVA

[00366] Mutant *Listeria* strains expressing a truncated form of a model antigen ovalbumin (OVA), the immunodominant epitope from mouse colorectal cancer (CT26) known as AH1 (SPSYVYHQF) (SEQ ID NO:55), and the altered epitope AH1-A5 (SPSYAYHQF (SEQ ID NO:56); Slansky *et al.*, 2000, *Immunity*, 13:529-538) were prepared. The pPL2 integrational vector (Lauer *et al.*, *J. Bacteriol.* 184:4177 (2002); U.S. Patent Publication No. 2003/0203472) was used to derive OVA and AH1-A5/OVA recombinant *Listeria* strains containing a single copy integrated into an innocuous site of the *Listeria* genome.

6.2.9. Construction of OVA-expressing *Listeria* (DP-L4056)

[00367] An antigen expression cassette consisting of hemolysin-deleted LLO fused with truncated OVA and contained in the pPL2 integration vector (pPL2/LLO-OVA) is first prepared. The *Listeria*-OVA vaccine strain is derived by introducing pPL2/LLO-OVA into the phage-cured *L. monocytogenes* strain DP-L4056 at the PSA (Phage from ScottA) attachment site $tRNA^{Arg}$-*attBB'*.

[00368] PCR is used to amplify the hemolysin-deleted LLO using the following template and primers:

Source: DP-L4056 genomic DNA

Primers:

Forward (*KpnI*-LLO nts. 1257-1276):

5'-CTCT<u>GGTACC</u>TCCTTTGATTAGTATATTC (SEQ ID NO:57)

(T_m: LLO-spec: 52°C. Overall: 80°C.)

Reverse (*BamHI-XhoI*-LLO nts. 2811-2792):

5'-CAAT<u>GGATCCCTCGAG</u>ATCATAATTTACTTCATCCC (SEQ ID NO:58)

(T_m: LLO-spec: 52°C. Overall: 102°C)

[00369] PCR is also used to amplify the truncated OVA using the following template and primers:

Source: pDP3616 plasmid DNA from DP-E3616 *E. coli* (Higgins et al., *Mol. Molbiol.* 31:1631-1641 (1999)).

Primers:

Forward (*XhoI- NcoI* OVA cDNA nts. 174-186):

5'-ATTTCTCGAGTCCATGGGGGTTCTCATCATC

(SEQ ID NO:59)

(T_m: OVA-spec: 60°C. Overall: 88°C.)

Reverse (*XhoI-NotI-HindIII*):

5'-GGTGCTCGAGTGCGGCCGCAAGCTT

(SEQ ID NO:60)

(T_m: Overall: 82°C.)

[00370] One protocol for completing the construction process involves first cutting the LLO amplicon with *KpnI* and *BamHI* and inserting the *KpnI/BamHI* vector into the pPL2 vector (pPL2-LLO). The OVA amplicon is then cut with *XhoI* and *NotI* and inserted into the pPL2-LLO which has been cut with *XhoI/NotI.* (Note: The pPL2 vector does not contain any *XhoI* sites; pDP-3616 contains one *XhoI* site, that is exploited in the OVA reverse primer design.) The construct pPL2/LLO-OVA is verified by restriction analysis (*KpnI*-LLO-*XhoI*-OVA-*NotI*) and sequencing. The plasmid pPL2/LLO-OVA is introduced into *E. coli* by transformation, followed by introduction and integration into *Listeria* (DP-L4056) by conjugation, exactly as described by Lauer et al. (or into another desired strain of *Listeria*).

6.2.10. Construction of Listeria strains expressing AH1/OVA or AH1-A5/OVA

[00371] To prepare *Listeria* expressing either the AH1/OVA or the AH1-A5/OVA antigen sequences, inserts bearing the antigen are first prepared from oligonucleotides and then ligated into the vector pPL2-LLO-OVA (prepared as described above).

[00372] The following oligonucleotides are used in preparation of the AH1 or AH1-A5 insert:

AH1 epitope insert (ClaI-PstI compatible ends):

Top strand oligo (AH1 Top):

5'-CGATTCCCCTAGTTATGTTTACCACCAATTTGCTGCA

(SEQ ID NO:61)

Bottom strand oligo (AH1 Bottom):

5'-GCAAATTGGTGGTAAACATAACTAGGGGAAT

(SEQ ID NO:62)

AH1-A5 epitope insert (ClaI-AvaII compatible ends):

[00373] The sequence of the AH1-A5 epitope is SPSYAYHQF (SEQ ID NO:56) (5'-AGT CCA AGT Tat GCA Tat CAT CAA TTT-3') (SEQ ID NO:63).

Top: 5'-**CGAT**AGTCCAAGTTATGCATATCATCAATTT**GC**
(SEQ ID NO:64)

Bottom: 5'-**GTCGC**AAATTGATGATATGCATAACTTGGACT**AT**
(SEQ ID NO:65)

[00374] The oligonucletide pair for a given epitope are mixed together at an equimolar ratio, heated at 95 °C for 5 min. The oligonucleotide mixture is then allowed to slowly cool. The annealed oligonucleotide pairs are then ligated at a 200 to 1 molar ratio with pPL2-LLO/OVA plasmid prepared by digestion with the relevant restriction enzymes. The identity of the new construct can be verified by restriction analysis and/or sequencing.

[00375] The plasmid can then be introduced into E. coli by transformation, followed by introduction and integration into *Listeria* (DP-L4056) by conjugation, exactly as described by Lauer et al., or into another desired strain of *Listeria*, such as an *actA*$^-$ mutant strain (DP-L0429), LLO L461T strain (DP-L4017), or *actA*$^-$/*inlB*$^-$ strain (DP-L4029*inlB*).

6.3. EXAMPLE 3: GENERATION OF MURINE TUMOR CELL LINES THAT EXPRESS HUMAN EphA2

6.3.1. Background

[00376] A mouse immunotherapy model was created for testing the Listeria-based vaccines of the invention. Three murine tumor cell lines, the CT26 murine colon carcinoma cell line, the B16F10 murine melanoma cell line, and the RenCa murine renal cell carcinoma cell line were created to express high levels of the huEphA2 protein. FACS cell sorting assays were performed to identify CT26, B16F10, and RenCa tumor cells expressing high levels of huEphA2, which were pooled and analyzed by Western blot analysis. Clones were further pooled by FACS cell sorting to generate subclones expressing the highest levels of huEphA2.

6.3.2. Selection of CT26 Murine Colon Carcinoma Cells Expressing High Levels of huEphA2

6.3.2.1. Transfection Assays With Lipofectamine™

[00377] CT26 cells were transfected with constructs containing huEphA2 using standard transfection techniques and commercially available Lipofectamine™ according to the manufacturer's instructions.

6.3.2.2. Flow Cytometry (FACS) Analysis

[00378] Single cell FACS sorting assays were performed by standard techniques to identify CT26 murine carcinoma tumor cell expressing high levels of human EphA2.

[00379] Figure 5 illustrates a representative experiment, showing that the EphA2-3 clone expressed the highest levels of human EphA2 protein.

6.3.2.3. Western Blot of Pooled Populations Expressing High Levels of huEphA2

[00380] Western blotting was also performed using standard techniques to determine the levels of human EphA2 protein expression in CT26 cells following FACS sorting of pooled populations of cells transfected with various constructs containing the huEphA2 gene. Figure 6 depicts results of a representative experiment. Compared to various clones tested, the huEphA2-3 clone expressed the highest levels of human EphA2 protein and was selected for the *in vivo* efficacy studies described below. Cells were further pooled to generate subclones expressing the highest levels of huEphA2.

6.3.3. Selection of B16F10 Murine Melanoma Cells Expressing High Levels of huEphA2

6.3.3.1. Retroviral Transduction

[00381] Human EphA2 was introduced into B16F10 murine melanoma cells by a retroviral transduction method to create clones expressing high levels of the protein.

6.3.3.2. Flow Cytometry (FACS) Analysis

[00382] As was performed on the CT26 cells, single cell FACS sorting assays were performed by standard techniques on B16F10 cells expressing huEphA2 to generate clones expressing high levels of huEphA2. Clones expressing the highest levels of huEphA2 were pooled and further examined by Western blot analysis. A representative FACS experiment is depicted in Figure 7, showing a B16F10 subclone expressing high levels of huEphA2.

6.3.3.3. Western Blot of Pooled Populations Expressing High Levels of huEphA2

[00383] Western blotting was also performed as described above to determine levels of huEphA2 protein expression in B16F10 cells following FACS sorting of pooled populations of cells containing the huEphA2 gene introduced by retroviral transduction. Cells were further pooled to generate subclones expressing the highest levels of huEphA2.

6.3.4. Selection of RenCa Murine Renal Cell Carcinoma Cells Expressing High Levels of huEphA2

6.3.4.1. Transfection Assays With LipofectamineTM

[0005] RenCa cells were transfected with constructs containing huEphA2 using standard transfection techniques and commercially available LipofectamineTM according to the manufacturer's instructions.

6.3.4.2. Flow Cytometry (FACS) Analysis

[0006] Single cell FACS sorting assays were performed by standard techniques to identify RenCa renal cell carcinoma tumor cells expressing high levels of human EphA2.

6.3.4.3. Western Blot of Pooled Populations Expressing High Levels of huEphA2

[00384] Western blotting was also performed using standard techniques to determine the levels of human EphA2 protein expression in RenCa cells following FACS sorting of pooled populations of cells transfected with various constructs containing the huEphA2 gene. Cells were further pooled to generate subclones expressing the highest levels of huEphA2.

6.3.5. Transfection of 293 Cells with pCDNA4 plasmids encoding full-length EphA2

[00385] Expression cassette constructs:

[00386] pCDNA4-EphA2

[00387] The native full-length EphA2 gene was cloned into the eukaryotic CMV promoter-based expression plasmid pCDNA4 (Invitrogen, Carlsbad, CA). Figure 8 shows the results of a Western blot analysis of lystates prepared from 293 cells transfected with the pCDNA4-EphA2 plasmid, and demonstrates the abundant expression in mammalian cells of full-length EphA2 protein.

6.4. EXAMPLE 4: Assessment of antigen-specific immune responses after vaccination

[00388] The vaccines of the present invention can be assessed using a variety of *in vitro* and *in vivo* methods. Some assays involve the analysis of antigen-specific T cells from the spleens of mice that have been vaccinated. For example C57Bl/6 or Balb/c are vaccinated by intravenous injection of 0.1 LD_{50} of a *Listeria* strain expressing OVA (or other appropriate antigen). Seven days after the vaccination, the spleen cells of the mice are harvested (typically 3 mice per group) by placing the spleens into ice cooled RPMI 1640 medium and preparing a single cell suspension from this. As an alternative, the lymph nodes of the mice could be similarly harvested, prepared as a single cell suspension and substituted for the spleen cells in the assays described below. Typically, spleen cells are

assessed for intraveneous or intraperitoneal administration of the vaccine while spleen cells and cells from lymph nodes are assessed for intramuscular, subcutaneous or intradermal administration of the vaccine.

[00389] Unless otherwise noted, all antibodies used in these examples can be obtained from Pharmingen, San Diego, CA.

6.4.1. ELISPOT Assay:

[00390] Using a *Listeria* strain having an OVA antigen as an example, the quantitative frequency of antigen-specific T cells generated upon immunization in a mouse model is assessed using an ELISPOT assay. The antigen-specific T cells evaluated are OVA specific CD8+ or LLO specific CD8+ or CD4+ T cells. This OVA antigen model assesses the immune response to a heterologous tumor antigen inserted into the vaccine and could be substituted with any antigen of interest. The LLO antigen is specific to *Listeria*. The specific T cells are assessed by detection of cytokine release (e.g. IFN-γ) upon recognition of the specific antigen. PVDF-based 96 well plates (BD Biosciences, San Jose, CA) are coated overnight at 4°C with an anti-murine IFN-γ monoclonal antibody (mAb R4; 5 μg/ml). The plates are washed and blocked for 2 hours at room temperature with 200 μL of complete RPMI. Spleen cells from vaccinated mice (or non vaccinated control mice) are added at 2 x 10^5 cells per well and incubated for 20 to 22 hours at 37°C in the presence of various concentrations of peptides ranging from 0.01 to 10 μM. The peptides used for OVA and LLO are either SL8, an MHC class I epitope for OVA, LLO_{190} (NEKYAQAYPNVS, Invitrogen) an MHC class II epitope for listeriolysin O (*Listeria* antigen), LLO_{296} (VAYGRQVYL), an MHC class I epitope for listeriolysin O, or LLO_{91} (GYKDGNEYI), an MHC class I epitope for listeriolysin O. LLO_{190} and LLO_{296} are used in a C57Bl/6 model, while LLO_{91} is used in a Balb/c model. After washing, the plates are incubated with secondary biotinylated antibodies specific for IFN-γ (XMG1.2) diluted in PBS to 0.5μg/ml. After incubation at room temperature for 2 hours, the plates are washed and incubated for 1 hour at 37 °C with a 1 nm gold goat anti-biotin conjugate (GAB-1; 1:200 dilution; Ted Pella, Redding, CA) diluted in PBS containing 1 % BSA. After thorough washing, the plates are incubated at room temperature for 2 to 10 minutes with substrate (Silver Enhancing Kit; 30 ml/well; Ted Pella) for spot development. The plates are then rinsed with distilled water to stop the substrate reaction. After the plates have been air-dried, spots in each well are counted using an automated ELISPOT plate reader (CTL, Cleveland, OH). The cytokine response is expressed as the number of IFN-γ spot-forming

cells (SFCs) per 2×10^5 spleen cells for either the OVA specific T cells or the *Listeria* specific T cells.

6.4.2. Intracellular Cytokine Staining Assay (ICS):

[00391] In order to further assess the number of antigen-specific CD8+ or CD4+ T cells and correlate the results with those obtained from ELISPOT assays, ICS is performed and the cells evaluated by flow cytometry analysis. Spleen cells from vaccinated and control groups of mice are incubated with SL8 (stimulates OVA specific CD8+ cells) or LLO_{190} (stimulates LLO specific CD4+ cells) for 5 hours in the presence of Brefeldin A (Pharmingen). The Brefeldin A inhibits secretion of the cytokines produced upon stimulation of the T cells. Spleen cells incubated with an irrelevant MHC class I peptide are used as controls. PMA (phorbol-12-myristate-13-acetate, Sigma) 20 ng/ml and ionomycin (Sigma) 2 μg/ml stimulated spleen cells are used as a positive control for IFN-γ and TNF-α intracellular cytokine staining. For detection of cytoplasmic cytokine expression, cells are stained with FITC-anti-CD4 mAb (RM 4-5) and PerCP-anti-CD8 mAb (53-6.7), fixed and permeabilized with Cytofix/CytoPerm solution (Pharmingen), and stained with PE-conjugated anti-TNF-α mAb (MP6-XT22) and APC-conjugated anti-IFN-γ mAb (XMG1.2) for 30 minutes on ice. The percentage of cells expressing intracellular IFN-γ and/or TNF-α was determined by flow cytometry (FACScalibur, Becton Dickinson, Mountain View, CA) and data analyzed using CELLQuest software (Becton Dickinson Immunocytometry System). As the fluorescent labels on the various antibodies can all be distinguished by the FACScalibur, the appropriate cells are identified by gating for those CD8+ and CD4+ that are stained with either or both of the anti-IFN-γ or anti-TNF-α.

6.4.3. Cytokine Expression of Stimulated Spleen Cells:

[00392] The level of cytokine secretion by the spleen cells of mice can also be assessed for control and vaccinated C57Bl/6 mice. Spleen cells are stimulated for 24 hours with SL8 or LLO_{190}. Stimulation with irrelevant peptide HSV-gB^2 (Invitrogen, SSIEFARL) is used as a control. The supernatants of the stimulated cells are collected and the levels of T helper-1 and T helper 2 cytokines are determined using an ELISA assay (eBiosciences, CO) or a Cytometric Bead Array Kit (Pharmingen).

6.4.4. Assessment of Cytotoxic T cell Activity:

[00393] The OVA specific CD8+ T cells can be further evaluated by assessing their cytotoxic activity, either *in vitro* or directly in C57Bl/6 mouse *in vivo*. The CD8+ T cells recognize and lyse their respective target cells in an antigen-specific manner. *In vitro*

cytotoxicity is determined using a chromium release assay. Spleen cells of naïve and *Listeria*-OVA (internal) vaccinated mice are stimulated at a 10:1 ratio with either irradiated EG7.OVA cells (EL-4 tumor cell line transfected to express OVA, ATCC, Manassas, VA) or with 100 nM SL8, in order to expand the OVA specific T cells in the spleen cell population. After 7 days of culture, the cytotoxic activity of the effector cells is determined in a standard 4-hour ^{51}Cr-release assay using EG7.OVA or SL8 pulsed EL-4 cells (ATCC, Manassas, VA) as target cells and EL-4 cells alone as negative control. The YAC-1 cell line (ATCC, Manassas, VA) is used as targets to determine NK cell activity, in order to distinguish the activity due to T cells from that due to NK cells. The percentage of specific cytotoxicity is calculated as 100 x (experimental release – spontaneous release) / (maximal release – spontaneous release). Spontaneous release is determined by incubation of target cells without effector cells. Maximal release is determined by lysing cells with 0.1% Triton X-100. Experiments are considered valid for analysis if spontaneous release is < 20% of maximal release.

[00394] For the assessment of cytotoxic activity of OVA-specific CD8+ T cells *in vivo*, spleen cells from naïve C57Bl/6 mice are split into two equivalent aliquots. Each group is pulsed with a specific peptide, either target (SL8) or control (HSV-gB^2), at 0.5 µg/ml for 90 minutes at 37 °C. Cells are then washed 3 times in medium, and twice in PBS + 0.1% BSA. Cells are resuspended at 1 x 10^7 per ml in warm PBS + 0.1% BSA (10 ml or less) for labeling with carboxyfluorescein diacetate succinimidyl ester (CFSE, Molecular Probes, Eugene, OR). To the target cell suspension, 1.25 µL of a 5mM stock of CFSE is added and the sample mixed by vortexing. To the control cell suspension, a ten-fold dilution of the CFSE stock is added and the sample mixed by vortexing. The cells are incubated at 37 °C for 10 minutes. Staining is stopped by addition of a large volume (>40 ml) of ice-cold PBS. The cells are washed twice at room temperature with PBS, then resuspended and counted. Each cell suspension is diluted to 50 x 10^6 per ml, and 100 µL of each population is mixed and injected via the tail vein of either naïve or vaccinated mice. After 12-24 hours, the spleens are harvested and a total of 5 x 10^6 cells are analyzed by flow cytometry. The high (target) and low (control) fluorescent peaks are enumerated, and the ratio of the two is used to establish the percentage of target cell lysis. The *in vivo* cytotoxicity assay permits the assessment of lytic activity of antigen-specific T cells without the need of *in vitro* re-stimulation. Furthermore, this assays assesses the T cell function in their native environment.

6.5. EXAMPLE 5: *IN VIVO* EphA2 EFFICACY STUDIES

6.5.1. Background

[00395] Efficacy studies were performed in mice inoculated with CT26 tumor cells expressing the extracellular domain (ED) of human EphA2 in order to characterize the anti-tumor effect of huEphA2. Endpoints measured were tumor volume and percent survival of the mice after tumor inoculation. The routes of inoculation were subcutaneous (s.c.) and intravenous (i.v.). HBSS and *Listeria* were administered as controls.

6.5.2. Control Vaccinations With AH1-A5-Expressing *Listeria*

[00396] Balb/c mice (n=5) were immunized with 0.1 LD_{50} *Listeria* 3 days post- i.v. inoculation of 1×10^5 CT26 cells. Figure 9A demonstrates that therapeutic immunization with *Listeria* expressing AH1-A5 increases survival of the inoculated animals. Figure 9B shows the result of a separate but otherwise equivalent experiment in which lungs of the experimental mice were harvested on Day 19 following cell inoculation and fixed. Gross assessment of lung nodules was also performed, demonstrating the absence of tumors in the lungs of test animals receiving *Listeria*-AH1/A5 as compared to control animals receiving a *Listeria* control.

6.5.3. Prophylactic EphA2 Vaccinations

6.5.3.1. Effect of Immunization with Listeria Expressing ECD of huEphA2 on CT26-hEphA2 Tumor Growth and Survival

[00397] Preventive studies were performed utilizing a pool of CT26 cells expressing huEphA2 generated by the single cell FACS assays described above. Groups of ten Balb/c mice per group were inoculated s.c. and groups of five mice per group were inoculated i.v. with CT26 colon carcinoma cells transfected with human EphA2 ("CT26-hEphA2"). The mice were immunized with 0.1 LD50 *Listeria* control or *Listeria* expressing the ECD of hEphA2 in a 200μl bolus. For the studies entailing s.c. inoculations with CD26, AH1/A5 *Listeria* were used as a positive control. The immunizations were performed 14 and 4 days prior to CT26-hEphA2 tumor challenge. Tumor volume measurements were obtained twice weekly for the course of the study to determine an anti-tumor effect of the vaccinations.

[00398] Figure 10A demonstrates the anti-tumor efficacy of *Listeria* expressing the ECD of hEphA2 against s.c. inoculations of huEphA2-expressing CT26 cells as compared to the negative controls (*p=0.0012). The data are summarized in Table 8 below:

Vaccination Group	Tumor Volume ($mm^3 \pm$ s.e.m.) (Day 42)	P vs. HBSS	P vs. *Listeria* Control
HBSS	1202.9 (± 321)	-	0.5528

Listeria Control	945.5 (± 338)	0.5528	-
Listeria-AH1/A5	392.5 (± 225)	0.0471	0.1895
Listeria-hEphA2-ECD	0.0 (± 0.0)	0.0012	0.0118

[00399] TABLE 8

[00400] Figure 10B demonstrates the anti-tumor efficacy of *Listeria* expressing the ECD of hEphA2 against i.v. inoculations of huEphA2-expressing CT26 cells as compared to the negative controls *p=0.0017). The data are summarized in Table 9 below:

Vaccination Group	Median Survival (Days)	P vs. HBSS	# Survivors (Day 65)
HBSS	18	-	0
Listeria Control	18	0.754	0
Listeria-AH1/A5	>65	0.0017	5
Listeria-hEphA2-ECD	>65	0.0017	3

[00401] TABLE 9

[00402] Preventive studies were performed according to the schedule described below. These studies utilized a pool of CT26 cells expressing huEphA2 generated by the single cell FACS assays described above.

[00403] **Groups:** Eight groups of ten mice per group. Groups 1-4 were inoculated s.c. and groups 5-8 were inoculated i.v. with CT26 colon carcinoma cells transfected with human EphA2, as shown in Table 10 below:

Treatment Group	**Number of Mice per Groups**
1. Control - HBSS	10
2. L4029 – control *Listeria monocytogenes*	10
3. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
4. L4029 – AH1 *Listeria monocytogenes*	10
5. Control - HBSS	10
6. L4029 – control *Listeria monocytogenes*	10
7. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
8. L4029 – AH1 *Listeria monocytogenes*	10

TABLE 10

[00404] **Schedule:** Animals received i.v. administrations of the agents listed above in 200μl bolus on Day 0 and Day 10. On Day 14, animals were inoculated with CT26 colon carcinoma cells transfected with human EphA2 (L4029EphA2-exFlag), *Listeria* control (L4029), or *Listeria* positive control containing the AH1 protein (L4029-AH1) (5 x10^5 cells in 100μl volume) either subcutaneously or intravenously (experimental lung metastases model). Tumor volume was measured bi-weekly (s.c inoculation only) and animal weights

assessed on a weekly basis. Any animals possessing tumors greater than 2000 mm^3 or demonstrating signs of morbidity (hunched posture, impaired breathing, decreases mobility, greater than 20% weight loss, *etc.*) were humanely euthanized. The experimental schedule is summarized in Table 11 below:

Group	Cell Inoculation Route (5 $\times 10^5$ cell in 100µl) (Day 14)	Primary Vaccination (Day 0)	Boost Vaccination (Day 10)
1. Control	s.c.	HBSS	HBSS
2. L4029	s.c.	2×10^7 CFU	2×10^7 CFU
3. L4029 EphA2-exFlag	s.c.	2×10^7 CFU	2×10^7 CFU
4. L4029 –AH1	s.c.	2×10^7 CFU	2×10^7 CFU
5. Control	i.v.	HBSS	HBSS
6. L4029	i.v.	2×10^7 CFU	2×10^7 CFU
7. L4029 EphA2-exFlag	i.v.	2×10^7 CFU	2×10^7 CFU
8. L4029 – AH1	i.v.	2×10^7 CFU	2×10^7 CFU

TABLE 11

[00405] In this study, vaccination with *Listeria*-huEphA2 exFlag demonstrated a significant anti-tumor effect in both the s.c. and experimental lung metastases models (i.v.). In the s.c. model, a significant reduction in tumor growth was achieved with 3 mice remaining tumor-free. This response was also specific compared to the control *Listeria* and vehicle treated animals. In the experimental lung metastases model, vaccination with *Listeria* huEphA2-exFlag also demonstrated efficacy compared to the vehicle treated group.

[00406] Figures 11A-11D illustrate results of the preventive experiments. Figure 11A shows that the tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 was significantly reduced when compared to vehicle (HBSS), *Listeria* (L4029) and *Listeria* positive (L4029-AH1) controls starting at day 21 and continued until day 32 post inoculation. Figure 11B also depicts results of the preventive experiments, showing again that the tumor volume of mice inoculated with CT26 cells expressing the ECD of huEphA2 (L4029-EphA2 exFlag) was significantly reduced when compared to the *Listeria* (L4029) control starting at day 21 and continued until day 32 post inoculation. Figure 11C illustrates the results of the prevention study in the s.c. model, measuring percent survival of the mice post CT26 tumor cell inoculation. Compared to all control groups, the L4029-EphA2 exFlag group had the most significant survival rate (indicated by triangles). Figure 11D illustrates the results of the prevention study in the lung metastases model, measuring the percent survival of the mice post tumor cell inoculation. Compared to all control groups, the L4029-EphA2 exFlag group had the most significant survival rate.

[00407] The foregoing data demonstrate that preventative immunization with *Listeria* expressing the ECD of hEphA2 suppresses CT26-hEphA2 tumor growth and increases survival.

6.5.3.2. Effect of Immunization with Listeria Expressing ICD of huEphA2 on the Survival of Mice Inoculated with RenCa-hEphA2

[00408] Preventive studies were performed utilizing a pool of RenCa cells (American Type Culture Collection, Manassas, VA) expressing huEphA2 generated and screened by the methods described above. Groups of ten Balb/c mice per group were inoculated subcutaneously with RenCa renal cell carcinoma cells expressing human EphA2 ("RenCa-hEphA2 cells"). The mice were immunized with 0.1 LD50 Listeria control or Listeria expressing the ICD of hEphA2 in a 200ml bolus. The immunizations were performed 18 and 4 days prior to RenCa-hEphA2 cell tumor challenge. Tumor volume measurements were obtained twice weekly for the course of the study to determine an anti-tumor effect of the vaccinations.

[00409] Figure 12 demonstrates the anti-tumor efficacy of Listeria expressing the ICD of hEphA2 against s.c. inoculations of huEphA2-expressing RenCA cells as compared to the negative controls. A significant anti-tumor response, as assessed by increased survival via Kaplan-Meier analysis, was observed in animals vaccinated with Listeria expressing the ICD of hEphA2 as compared to animals vaccinated with Listeria alone (*p=0.0079).

6.5.4. Therapeutic EphA2 Vaccinations

[00410] Therapeutic studies were performed utilizing a pool of CT26 cells expressing huEphA2 generated by the single cell FACS assays described above.

[00411] A representative therapeutic study was performed as follows:

[00412] **Groups:** Six groups of ten mice per group. Groups 1-3 were inoculated s.c. and groups 4-6 were inoculated i.v. with CT26 murine colon carcinoma cells, as shown in Table 12 below:

Treatment Group	Number of Mice per Groups
1. Control - HBSS	10
2. L4029 – control *Listeria monocytogenes*	10
3. L4029-EphA2 exFlag – *Listeria monocytogenes* expressing extracellular domain of human EphA2	10
4. Control - HBSS	10
5. L4029 – control *Listeria monocytogenes*	10
6. L4029-EphA2 exFlag – *Listeria monocytogenes*	10

expressing extracellular domain of human EphA2	

TABLE 12

[00413] **Schedule**: Animals were inoculated with CT26 colon carcinoma cells transfected with human EphA2 (L4029-EphA2 exFlag), *Listeria* control (L4029-control) or vehicle (HBSS) (5 x10^5 cells in 100ml volume) either subcutaneously or intravenously (experimental lung metastases model). Three days after cell inoculation, animals received i.v. administrations of the agents listed above in 200ml bolus. Two weeks following the first administration, the animals received a booster vaccination. Tumor volume was measured bi-weekly (s.c inoculation only) and animal weights assessed on a weekly basis. Any animals possessing tumors greater than 2000 mm^3 or demonstrating signs of morbidity (hunched posture, impaired breathing, decreases mobility, greater than 20% weight loss, etc.) were humanely euthanized. The schedule is summarized in Table 13 below.

Group	**Cell Inoculation Route** (5 x10^5 cell in 100μl)	**Primary Vaccination** (Day 3)	**Boost Vaccination** (Day 17)
1. Control	s.c.	HBSS	HBSS
2. L4029	s.c.	6x10^6 to 2x10^7 CFU	6x10^6 to 2x10^7 CFU
3. L4029 EphA2-exFlag	s.c.	6x10^6 to 2x10^7 CFU	6x10^6 to 2x10^7 CFU
4. Control	i.v.	HBSS	HBSS
5. L4029	i.v.	6x10^6 to 2x10^7 CFU	6x10^6 to 2x10^7 CFU
6. L4029 EphA2-exFlag	i.v.	6x10^6 to 2x10^7 CFU	6x10^6 to 2x10^7 CFU

TABLE 13

[00414] Figures 13A-13C illustrate the results of a typical therapeutic study. In Figure 13A, tumor volume was measured at several intervals post inoculation. Compared to the HBSS and Listeria controls, the mice inoculated with CT26 cells expressing the ECD of huEphA2 had a significantly lower tumor volume after day 14 and continued onto day 28. Figure 13B depicts the mean tumor volume of mice inoculated with CT26 cells containing either Listeria control or huEphA2. Compared to control, the mice inoculated with CT26 cells expressing huEphA2 had a reduced mean tumor volume. Figure 13C represents the results of the therapeutic study using the lung metastases model, measuring percent survival of the mice post inoculation with CT26 cells with either HBSS or Listeria control, or Listeria expressing the ECD of huEphA2. Animals inoculated with CT26 cells expressing the ECD of huEphA2 (depicted by triangles) showed a higher percent survival rate compared to controls.

[00415] In another study, groups of ten Balb/c mice per group were inoculated s.c. or i.v. with CT26 colon carcinoma cells transfected with human EphA2 ("CT26-hEphA2"). The mice were immunized with 0.1 LD_{50} actA *Listeria* control or *Listeria* expressing the ICD of hEphA2 in a 200μl bolus. In one regimen, the immunizations were performed 6 and 14 days post s.c. CT26-hEphA2 tumor inoculation. In another regimen, the immunizations were performed 3 and 14 days post i.v. CT26-hEphA2 tumor inoculation. Anti-tumor efficacy was determined from twice weekly tumor measurements and survival.

[00416] Significant anti-tumor efficacy was observed in the *Listeria*-hEphA2 vaccinated animals (p=0.0035).

[00417] Figure 14A demonstrates the tumor measurements of immunized animals. This data is summarized in Table 14 below:

Vaccination Group	Tumor Volume (mm^3 ± s.e.m.) (Day 21)	P vs. HBSS	P vs. *Listeria* Control
HBSS	1827 (± 518)	-	0.961
Listeria Control	1799 (± 267)	0.961	-
Listeria-AH1/A5	0	0.0005	0.000003
Listeria-hEphA2-ICD-1	694 (± 232)	0.0054	0.006
Listeria-hEphA2-ICD-2	731 (± 176)	0.052	0.004

[00418] **TABLE 14**

[00419] Figure 14B demonstrates the survival time of immunized animals. This data is summarized in Table 15 below:

Vaccination Group	Median Survival (Days)	P vs. HBSS	# Survivors (Day 65)
HBSS	19	-	0
Listeria Control	20	Ns	0
Listeria-hEphA2-ICD-1	>65	0.0035	3
Listeria-hEphA2-ICD-2	>65	0.0035	4
Listeria-hEphA2-ICD-3	>65	0.0035	4

[00420] **TABLE 15**

[00421] Immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with recombinant *Listeria* encoding OVA.AH1 (MMTV gp70 immunodominant epitope) or OVA.AH1-A5 (MMTV gp70 immunodominant epitope, with heteroclitic change for enhanced T-cell receptor binding) confers long-term survival (Figure 14C).

[00422] The EphA2 CO domain is strongly immunogenic, and a significant long term increase in survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors was observed when immunized with recombinant *Listeria* encoding codon-optimized or native EphA2 CO domain sequence (Figure 14D).

[00423] The EphA2 EX2 domain is poorly immunogenic, and increased survival of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors was observed only when immunized with recombinant *Listeria* encoding codon-optimized secA1 signal peptide fused with the codon-optimized EphA2 EX2 domain sequence. Therapeutic efficacy was not observed in mice when immunized with recombinant *Listeria* encoding native secA1 signal peptide fused with the codon-optimized EphA2 EX2 domain sequence (Figure 14E). The desirability of using both codon-optimized secA1 signal peptide and EphA2 EX2 domain sequences was supported by statistically significant therapeutic anti-tumor efficacy, as shown in the table below:

[00424] A comparison by log-rank test of survival curves shown in Figure 14E and summarized in Table 16 below:

Experimental Group	Median Survival (Days)	Significance versus HBSS cohort (p value)	Significance versus actA-native secA1/EphA2 EX2 cohort (p value)
HBSS	19	-	-
ActA	20	NS	NS
actA-native secA1-EphA2 EX2 (native)	19	NS	-
actA-native secA1-EphA2 EX2 (CodOp)	24	0.0035	NS
actA-CodOp secA1-EphA2 EX2 (CodOp)	37	0.0035	0.0162
actA-native secA1-EphA2 CO (CodOp)	>99	0.0035	0.0015

TABLE 16

[00425] Significantly, even though pCDNA4-EphA2 plasmid transfected 293 cells yielded very high levels of protein expression, immunization of Balb/C mice bearing CT26.24 (huEphA2+) lung tumors with the pCDNA4-EphA2 plasmid did not result in any observance of therapeutic anti-tumor efficacy (Figure 14F).

[00426] For therapeutic *in vivo* tumor studies, female Balb/C mice were implanted IV with 5 x 10^5 CT26 cells stably expressing EphA2. Three days later, mice were randomized and vaccinated IV with various recombinant *Listeria* strains encoding EphA2. In some cases (noted in figures) mice were vaccinated with 100 μg of pCDNA4 plasmid or pCDNA4-EphA2 plasmid in the *tibialis* anterior muscle. As a positive control, mice were vaccinated IV with recombinant *Listeria* strains encoding OVA.AHI or OVA.AH1-A5 protein chimeras. Mice were vaccinated on days 3 and 14 following tumor cell implantation. Mice injected with Hanks Balanced Salt Solution (HBSS) buffer or unmodified *Listeria* served as negative controls. All experimental cohorts contained 5

mice. For survival studies mice were sacrificed when they started to show any signs of stress or labored breathing.

[00427] The foregoing data demonstrate that therapeutic immunization with *Listeria* expressing the hEphA2 suppresses established CT26-hEphA2 tumor growth and increases survival.

6.6. EXAMPLE 6: Long-term Suppression of CT26-hEphA2 Tumor Growth Upon Rechallenge

[00428] Balb/c mice failing to form tumors after preventative immunization with *Listeria* expressing either the ICD or ECD of hEphA2 against CT26-hEphA2 tumor challenged, were re-challenged (s.c.) with both CT26 parental cell line and CT26-hEphA2 cells on opposite flanks 56 days after initial tumor challenge and 60 days after the last immunization. Age-matched mice were used as a control in this experiment.

[00429] Re-challenge with parental CT26 cells showed no statistically significant differences in tumor growth between groups (data not shown). However, as shown in Figure 15, both groups vaccinated with *Listeria* expressing either the ICD or ECD of hEphA2 demonstrated a significant suppression of tumor growth upon re-challenge (*$p<0.041$).

6.7. EXAMPLE 7: Immunization with Listeria Expressing hEphA2 Elicits an EphA2-Specific CD8+ T Cell Response

[00430] Balb/c mice (n=3) were immunized with *Listeria* L461T expressing the intracellular domain of hEphA2 (hEphA2-ICD) or *ΔactA* expressing codon optimized extracellular domain of hEphA2 (hEphA2-ECD) two weeks apart. Mice were euthanized, and spleens harvested and pooled 6 days after the last immunization. For the ELISPOT assay, the cells were re-stimulated *in vitro* with P815 cells expressing full-length hEphA2 or cell lysates prepared from these cells. The parental P815 cells or cell lysates served as a negative control. Cells were also stimulated with recombinant hEphA2 Fc fusion protein. IFN-gamma positive spot forming colonies (SFCs) were measured using a 96 well spot reader.

[00431] As shown in Figure 16, increased IFN-gamma SFCs were observed with spleen cells derived from mice vaccinated with Listeria-hEphA2. Both hEphA2 expressing cells or cell lysates stimulation resulted in an increase in IFN-gamma SFC which suggests an EphA2-specific CD8+ as well as CD4+ T cell response. Spleen cells from mice vaccinated with the parental *Listeria* control did not demonstrate an increase in IFN-gamma SFC.

6.8. EXAMPLE 8: Both CD4+ and CD8+ T Cell Responses are Required for Maximal hEphA2-Directed Anti-Tumor Efficacy

[00432] Balb/c mice (n=10) were inoculated i.v. with 2 x 10^5 CT26-hEphA2 on day 0. CD4+ cells and CD8+ T-cells were depleted by injecting 200 μg anti-CD4 (ATCC hybridoma GK1.5) or anti-CD8 (ATCC hybridoma 2.4-3) on Days 1 and 3, which was confirmed by FACS analysis (data not shown). Mice were then immunized i.v. with 0.1 LD_{50} *Listeria* L461T expressing hEphA2 ICD on Day 4 and monitored for survival.

[00433] As shown in Figure 17, both CD4+ and CD8+ depleted groups failed to demonstrate the degree of anti-tumor response seen in the non-T cell depleted animals. The data are summarized in Table 17 below:

Vaccination Group	Median Survival (Days)	P vs. HBSS	# Survivors (Day 67)
HBSS	17	-	0
Listeria-hEphA2-ICD	>67	<0.0001	7
Listeria-hEphA2-ICD + anti-CD4	19	0.03	2
Listeria-hEphA2-ICD + anti-CD8	24	0.0002	0

[00434] **TABLE 17**

[00435] The foregoing data indicate a requirement for both CD4+ and CD8+ T cells in optimal suppression of tumor growth.

6.9. EXAMPLE 9: Therapeutic Vaccination with Listeria Expressing Human EphA2 ICD Enhances CD45+ Tumor Infiltrate

[00436] Balb/c mice (n=3) were immunized with 0.1 LD50 actA-Listeria control or Listeria expressing either the ECD or ICD of hEphA2, 6 days post s.c. CT26-hEphA2 tumor inoculation. 9 days post-vaccination, tumors were harvested, fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 4 μm. Microscope slides were prepared from the tumor sections. The tissues on the slides were deparaffinized and rehydrated as follows: 4 changes with xylene, 5 minutes each; 2 changes with absolute alcohol, 5 minutes each; 1 change with 95% alcohol for 5 minutes; 1 change with 70% for 5 minutes; and two changes with distilled water.

[00437] Steam antigen retrieval was performed in a Black and Decker Rice steamer using target antigen retrieval (TAR) solution (DakoCytomation, Carpinteria, CA) using a modification of the manufacture's protocol. The slides were placed into TAR solution preheated to just below boiling temperature and incubated for 20 minutes. The slides were

then removed from the TAR solution and allowed to cool at room temperature for 20 minutes, and rinsed twice in TBS assay buffer.

[00438] Staining of the slides with biotinylated antibody was performed as follows:

[00439] Endogenous peroxidase was blocked by immersing the slides in solution of 3% hydrogen peroxide in methanol, for 10 minutes, followed with 2 changes of distilled water, 5 minutes each. Protein was blocked by immersing the slides in a solution of 5% Bovine Serum Albumin (BSA) in 1x Tris buffered saline with 0.01% Tween 20 (TBST) for at least 30 minutes.

[00440] After wiping excess BSA solution from the slide, creating a "pool", centered around tissue, the slide was laid flat in humid chamber and biotinylated rat anti-mouse CD45/B220 (Pharmingen) at 1:100 dilution in a solution of 1%BSA/TBST was applied. The slide was incubated in a humid chamber overnight at room temperature with care taken to prevent drying of the tissue sections.

[00441] The next morning, the slides are washed with 2 changes of TBST, the second one lasting 10 minutes. Streptavidin conjugated with either HRP or AP is applied, incubating for 30 minutes at room temp. The slides are washed with two changes of TBST, visualized with an appropriate substrate chromagen (for Strep-HRP, DAB is used). After a wash in distilled water, the slides are counterstained with Mayers Hematoxylin by immersing the slides in dye for 2 minutes. The slides are then washed in running tap water until water runs clear, immersed in bluing agent (Scotts substitute tap water) for 30 seconds, and washed again in tap water. The slides are dehydrated and cleared in graded alcohols through xylene (or xylene substitute) by the following washes: 95% alcohol for 1 minute, 3 changes absolute alcohol for 1 minute each, and 4 changes xylenes for 1 minute each.

[00442] Mounting media is applied to the cover slips (for xylene, DPX mountant is used) and the slides are allowed to dry over night prior to visualization.

[00443] The sections were visualized on a Nikon Eclipse E400 and images captured with a Nikon DXM1200 digital camera (Figure 18A). Data was further normalized to tumor volume (Figure 18B).

[00444] The results demonstrate that tumor associated infiltrating lymphocytes are increased following therapeutic vaccination.

7. Equivalents

[00445] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00446] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

We claim:

1. A method of eliciting an immune response against an EphA2-expressing cell in a subject, said method comprising administering to a subject a composition comprising a *Listeria* bacterium that expresses an EphA2 antigenic peptide in an amount effective to elicit an immune response against an EphA2-expressing cell.

2. The method of claim 1, wherein the *Listeria* is *Listeria monocytogenes*.

3. The method of claim 2, wherein the *Listeria* is attenuated.

4. The method of claim 1, wherein the nucleic acid encoding the EphA2 antigenic peptide comprises a nucleotide sequence encoding a secretory signal operatively linked to the sequence encoding the EphA2 antigenic peptide.

5. A method of claim 1, wherein the subject has cancer.

6. The method of claim 5, wherein said cancer is of an epithelial cell origin.

7. The method of claim 5, wherein said cancer is of a T cell origin.

8. The method of claim 6, wherein said cancer is cancer of the skin, lung, colon, breast, prostate, bladder or pancreas or is a renal cell carcinoma or melanoma.

9. The method of claim 7, wherein said cancer is a leukemia or a lymphoma.

10. The method of claim 1, wherein the subject has a non-neoplastic hyperproliferative disorder.

11. The method of claim 10, wherein the hyperproliferative disorder is an epithelial cell disorder.

12. The method of claim 11, wherein the hyperproliferative disorder is asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis.

13. A method of treating a human subject having a hyperproliferative disorder of EphA2-expressing cells, said method comprising administering to the subject a composition comprising an EphA2 antigenic peptide-expressing *Listeria* bacterium in an amount effective to treat a hyperproliferative disorder of EphA2-expressing cells.

14. The method of claim 13, wherein the *Listeria* is *Listeria monocytogenes*.

15. The method of claim 13, wherein the subject has cancer.

16. The method of claim 15, wherein the cancer is of an epithelial cell origin.

17. The method of claim 15, wherein the cancer is of an endothelial cell origin.

18. The method of claim 15, wherein the cancer is of a T cell origin.

19. The method of claim 15, wherein said cancer comprises cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells.

20. The method of claim 16, wherein said cancer is cancer of the skin, lung, colon, breast, prostate, bladder or pancreas or is a renal cell carcinoma or melanoma.

21. The method of claim 18, wherein said cancer is a leukemia or a lymphoma.

22. The method of claim 13, wherein the subject has a non-neoplastic hyperproliferative disorder.

23. The method of claim 22, wherein the hyperproliferative disorder is an epithelial cell disorder.

24. The method of claim 23, wherein the hyperproliferative disorder is asthma, chronic pulmonary obstructive disease, lung fibrosis, bronchial hyper responsiveness, psoriasis, and seborrheic dermatitis.

25. The method of claim 1 or 13, wherein the EphA2 polypeptide comprises full length EphA2.

26. The method of any one of claims 1 and 13, wherein the EphA2 polypeptide comprises the extracellular domain of EphA2.

27. The method of any one of claims 1 and 13, wherein the EphA2 polypeptide is a chimeric polypeptide comprising at least an antigenic portion of EphA2 and a second polypeptide.

28. The method of claim 1 or 13, wherein the composition comprises a plurality of EphA2 antigenic peptide-expressing *Listeria*.

29. The method of claim 1 or 13, wherein the EphA2 antigenic peptide-expressing *Listeria* expresses a plurality of EphA2 antigenic peptides.

30. The method of any one of claims 1 and 13, further comprising administering an additional anti-cancer therapy.

31. The method of claim 30, wherein the additional anti-cancer therapy is an agonistic EphA2 antibody.

32. The method of claim 30, wherein the additional anti-cancer therapy is an anti-idiotype of an agonistic EphA2 antibody.

33. The method of claim 30, wherein the additional anti-cancer therapy is chemotherapy, biological therapy, immunotherapy, radiation therapy, hormonal therapy, or surgery.

34. The method of any one of claims 1 and 13, wherein said administering is mucosal, parenteral, intramuscular, intraperitoneal, intravenous or oral.

35. The method of claim 1 or 13, wherein the administration elicits a $CD4^+$ T-cell response, a $CD8^+$ T-cell response, an innate immune response, an antibody response, or a combination of one or more of the foregoing.

36. The method of claim 35, wherein the administration elicits both a $CD4^+$ T-cell response and a $CD8^+$ T-cell response.

37. A method of treating a human subject having a disease involving aberrant angiogenesis, said method comprising administering to the subject a composition comprising an EphA2 antigenic peptide-expressing *Listeria* bacterium in an amount effective to treat disease involving aberrant angiogenesis.

38. The method of claim 1, wherein the subject has a disease involving aberrant angiogenesis.

39. The method of claim 37 or 38, wherein the disease is macular degeneration, diabetic retinopathy, retinopathy of prematurity, vascular restenosis, infantile hemangioma, verruca vulgaris, psoriasis, Kaposi's sarcoma, neurofibromatosis, recessive dystrophic epidermolysis bullosa, rheumatoid arthritis, ankylosing spondylitis, systemic lupus, psoriatic arthropathy, Reiter's syndrome, and Sjogren's syndrome, endometriosis, preeclampsia, atherosclerosis or coronary artery disease.

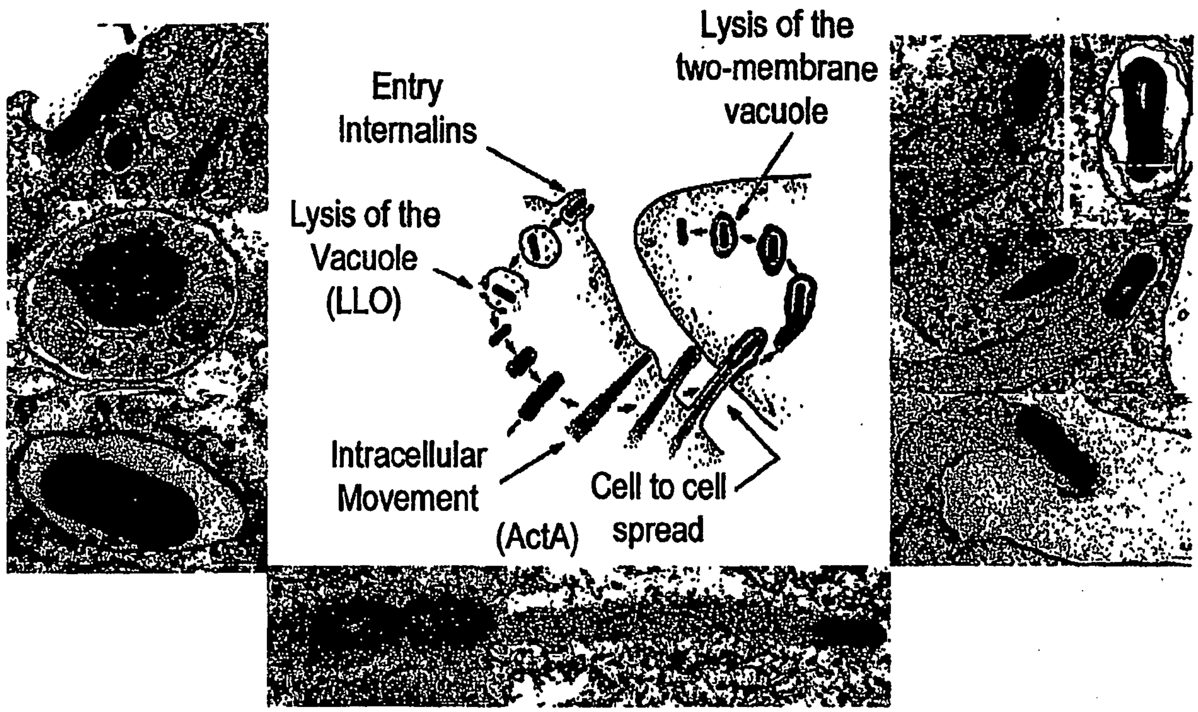

Lysis of the
two-membrane
vacuole
Entry
Internalins
Lysis of the
Vacuole
(LLO)
Intracellular
Movement
(ActA)
Cell to cell
spread

FIG.1A

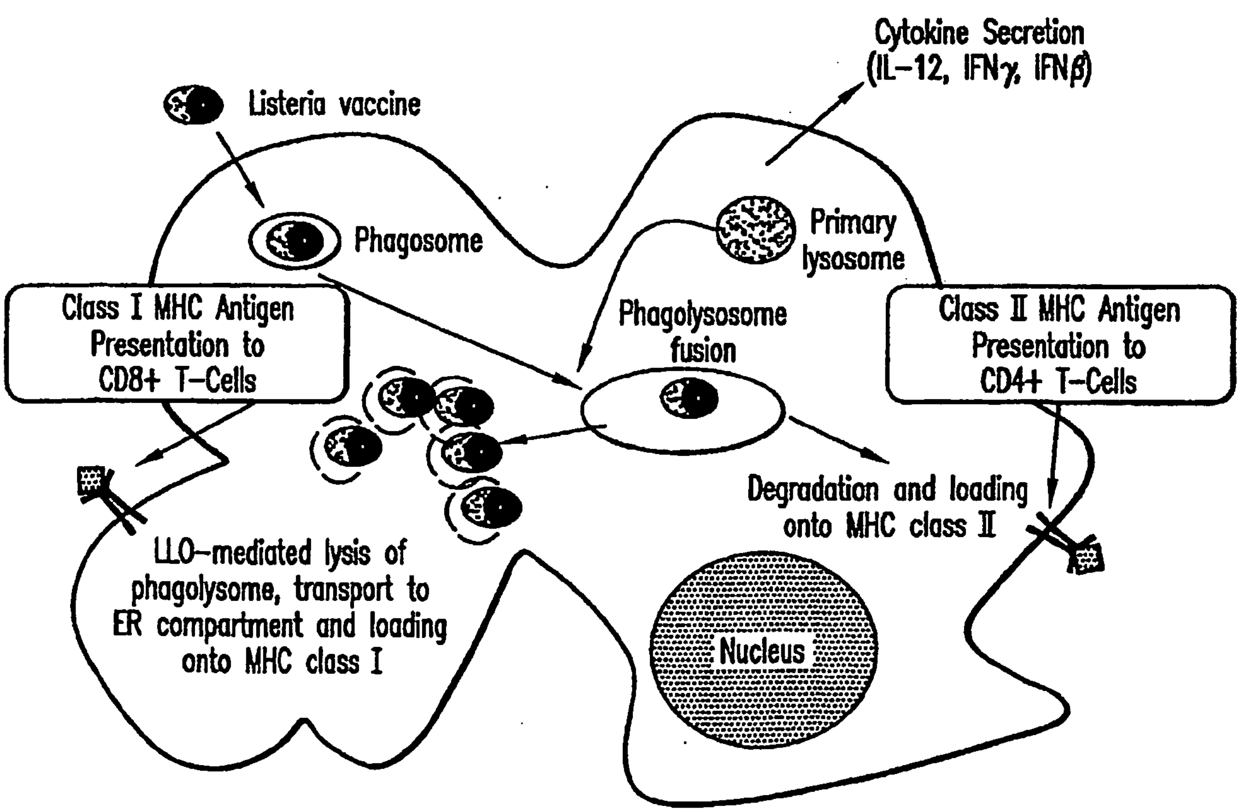
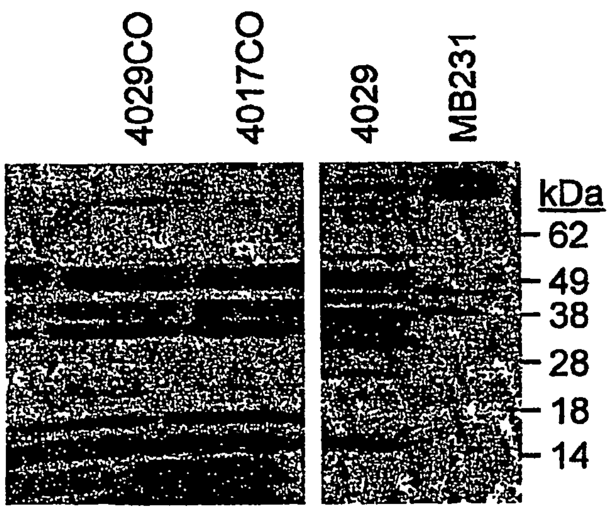

Cytokine Secretion
(IL-12, IFNγ, IFNβ)
Listeria vaccine
Phagosome
Primary
lysosome
Class I MHC Antigen
Presentation to
CD8+ T-Cells
Phagolysosome
fusion
Class II MHC Antigen
Presentation to
CD4+ T-Cells
Degradation and loading
onto MHC class II
LLO-mediated lysis of
phagolysome, transport to
ER compartment and loading
onto MHC class I
Nucleus

FIG.1B



Expected mass of EphA2 proteins

Full-length: 114 kDa

EphA2 CO: 52 kDa

EphA2 EX2: 62 kDa

Ab: Rabbit anti-EphA2 (carboxy terminus-specific) polyclonal antibody

FIG.2

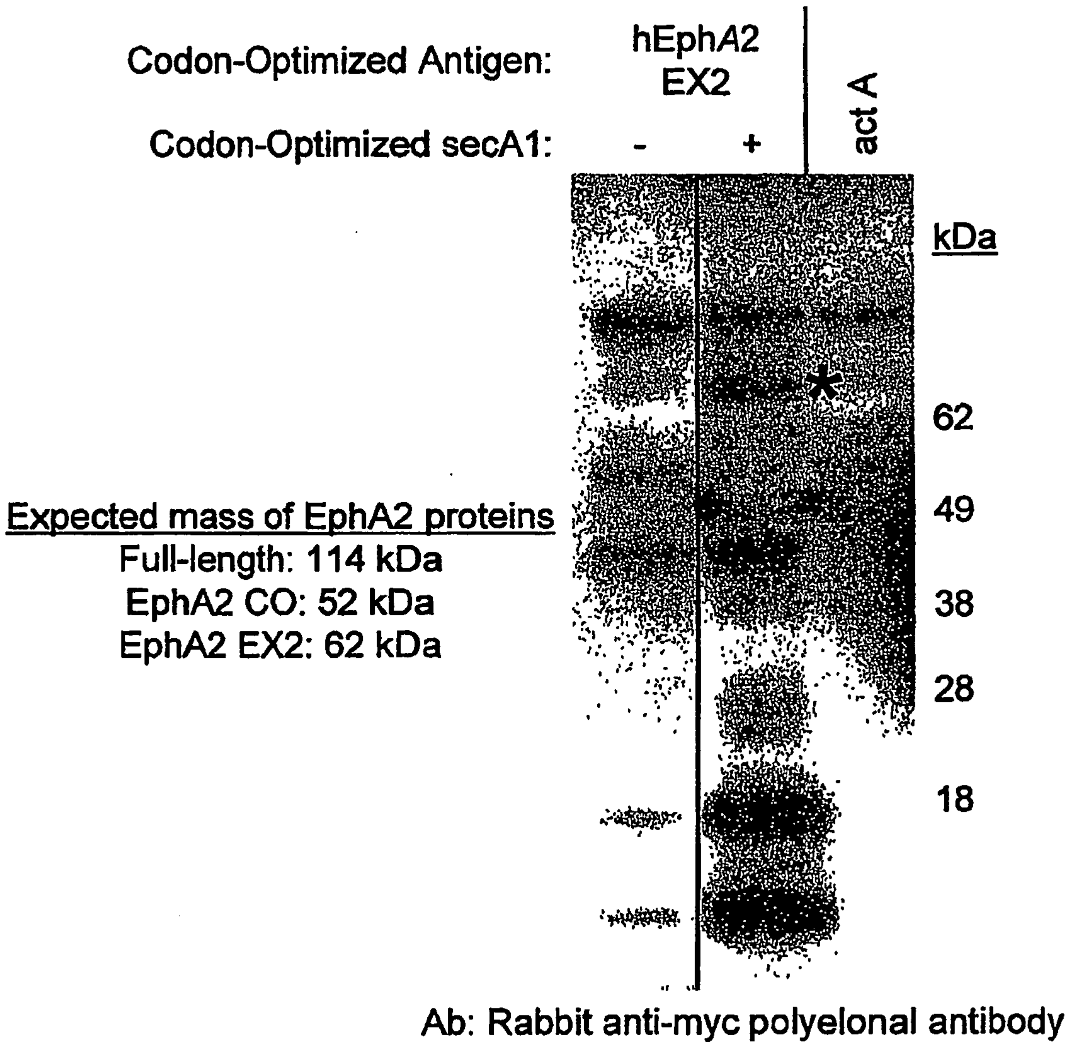
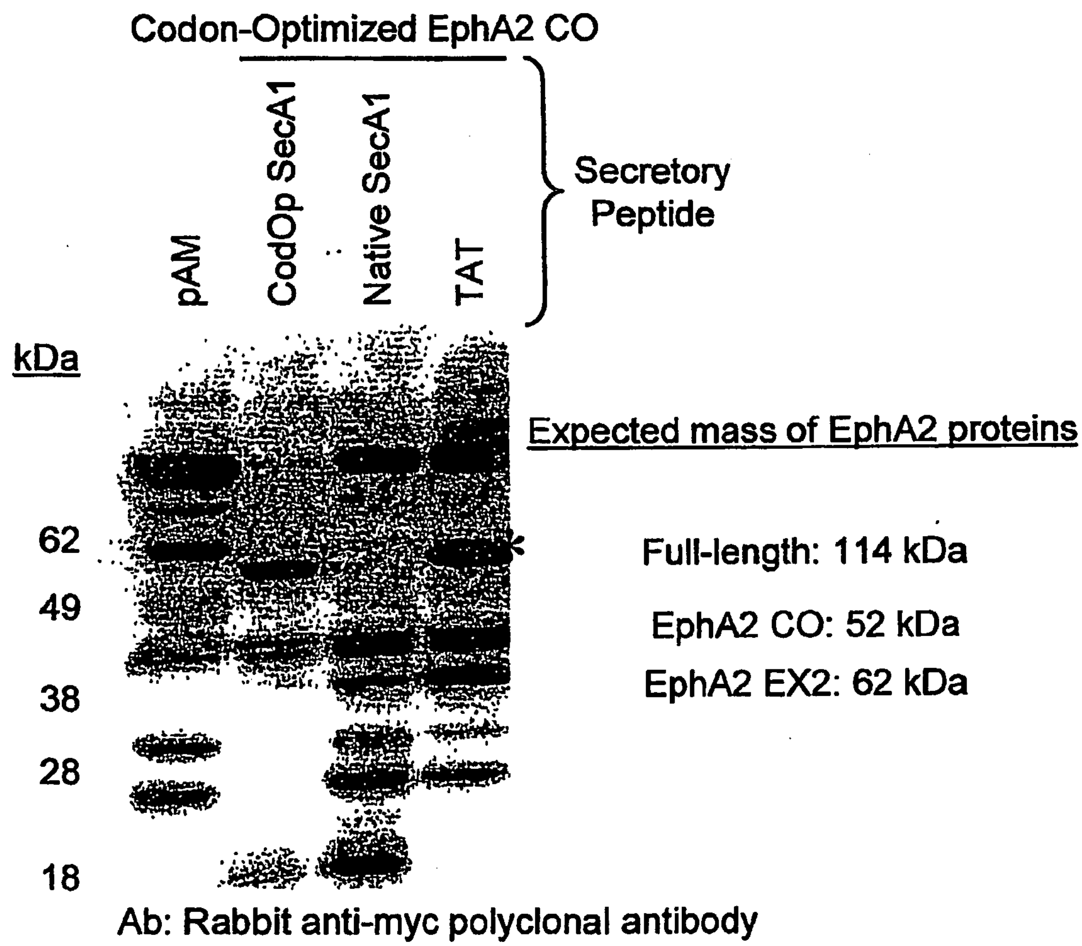

Codon-Optimized Antigen:
hEphA2
EX2
act A
Codon-Optimized secA1:
-
+
kDa
62
49
38
28
18
*
Expected mass of EphA2 proteins
Full-length: 114 kDa
EphA2 CO: 52 kDa
EphA2 EX2: 62 kDa
Ab: Rabbit anti-myc polyelonal antibody

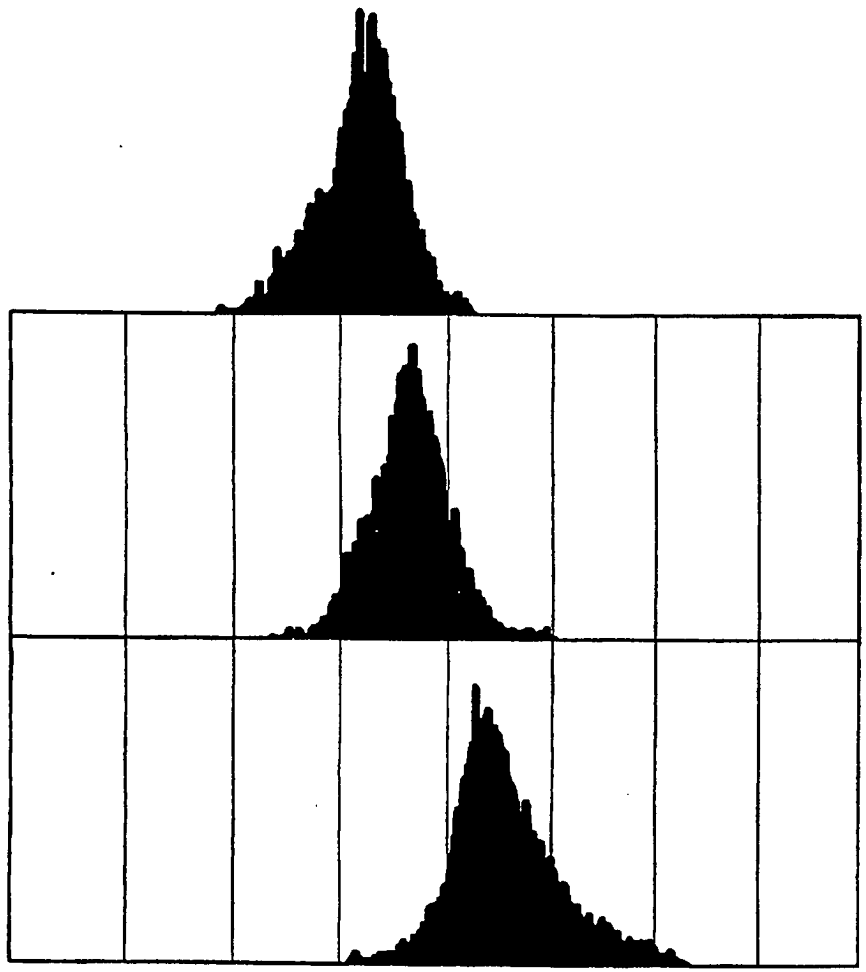
FIG.3



Codon-Optimized EphA2 CO
pAM
CodOp SecA1
Native SecA1
TAT
Secretory Peptide
kDa
62
49
38
28
18
Expected mass of EphA2 proteins
Full-length: 114 kDa
EphA2 CO: 52 kDa
EphA2 EX2: 62 kDa
Ab: Rabbit anti-myc polyclonal antibody

FIG.4

Human EphA2 Expression in CT26 Subclones
Generation of "the Super Clone"



A8–B5 (mean = 45.0)
Average

B7–D10 (mean = 73.18)
Medium

B7–E6 (mean = 233.0)
Super Clone!

• Subclones of transfected clones obtained by FACS sorting into 96 well plates

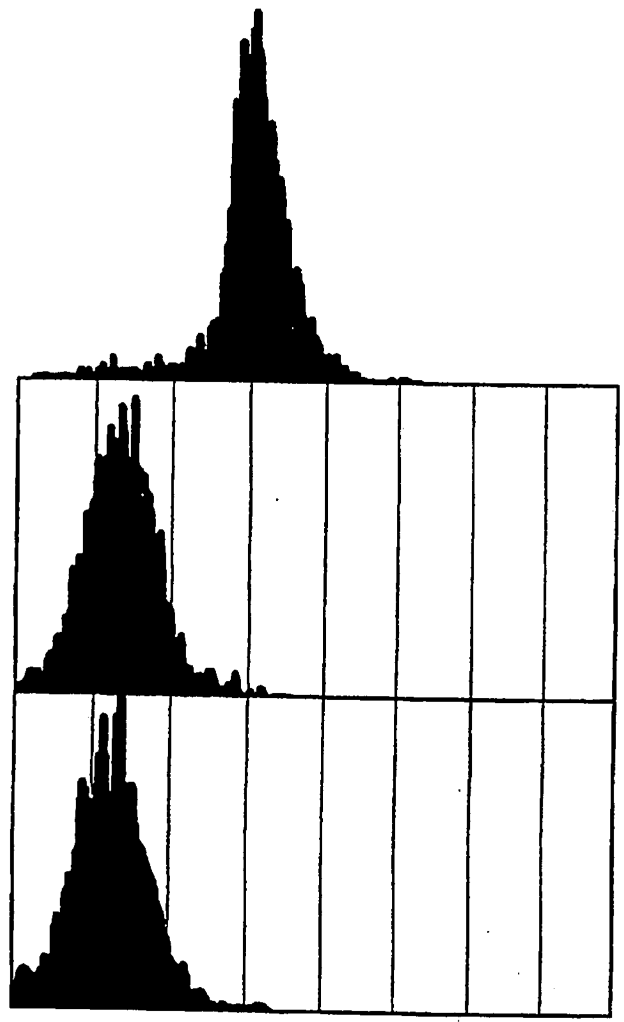
FIG. 5

Human EphA2 Protein Expression in CT26 Murine Colon Carcinoma Cells Following FACS Sorting

pooled populations of transfected cells sorted by FACS

FIG.6

Human EphA2 Protein Expression in B16F10 Cells
Round 3

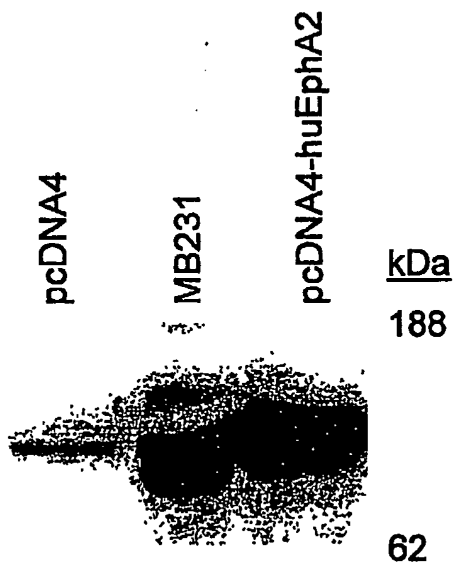


Anti–EphA2 + 2°(mean = 42.4)

2°–control (mean = 5.58)

Unstained (mean = 4.73)

FIG.7



Expected mass of EphA2 proteins

Full-length: 114 kDa

EphA2 CO: 52 kDa

EphA2 EX2: 62 kDa

Ab: Rabbit anti-EphA2 (carboxy terminus-specific) polyclonal antibody

FIG.8

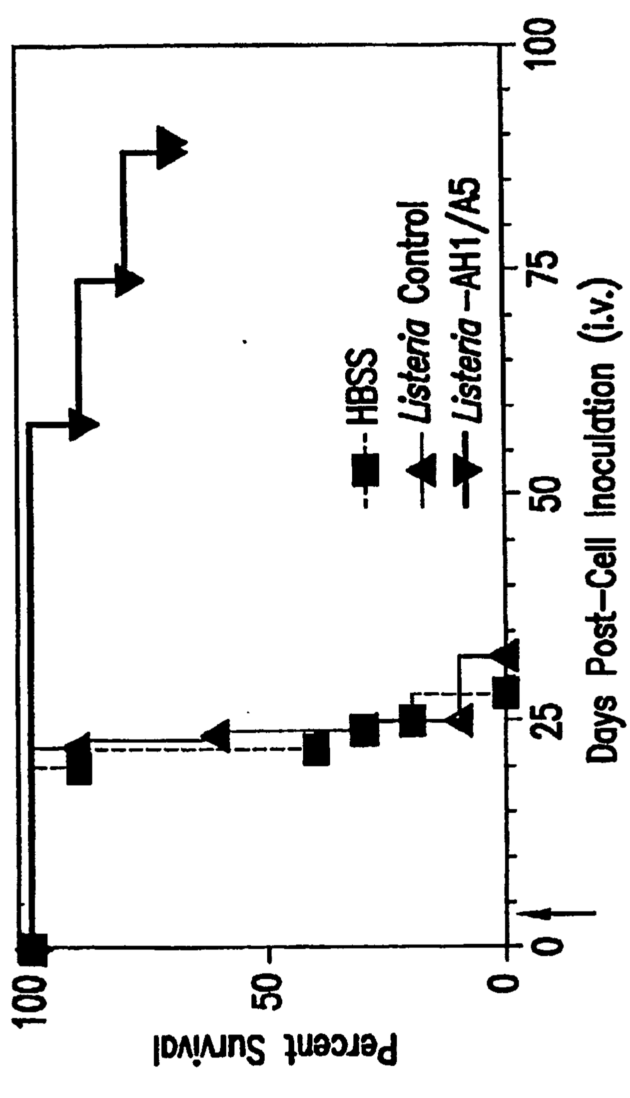

Percent Survival
100
50
0
0
25
50
75
100
Days Post-Cell Inoculation (i.v.)
HBSS
Listeria Control
Listeria-AH1/A5

FIG.9A


Listeria Control
Listeria-AH1/A5

FIG.9B

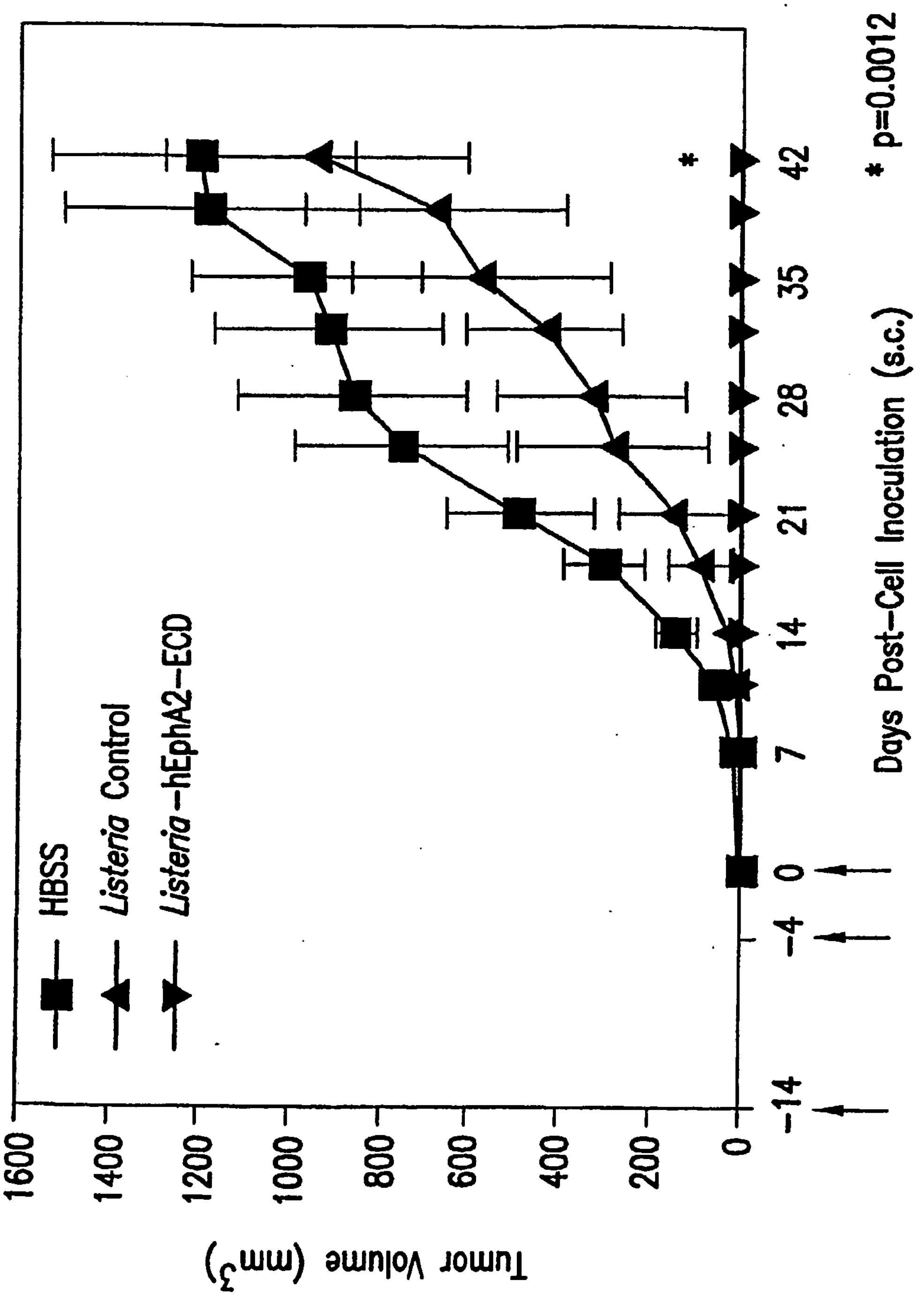

HBSS
Listeria Control
Listeria–hEphA2–ECD
Tumor Volume (mm^3)
1600
1400
1200
1000
800
600
400
200
0
–14
–4
0
7
14
21
28
35
42
Days Post–Cell Inoculation (s.c.)
*
* p=0.0012

FIG.10A

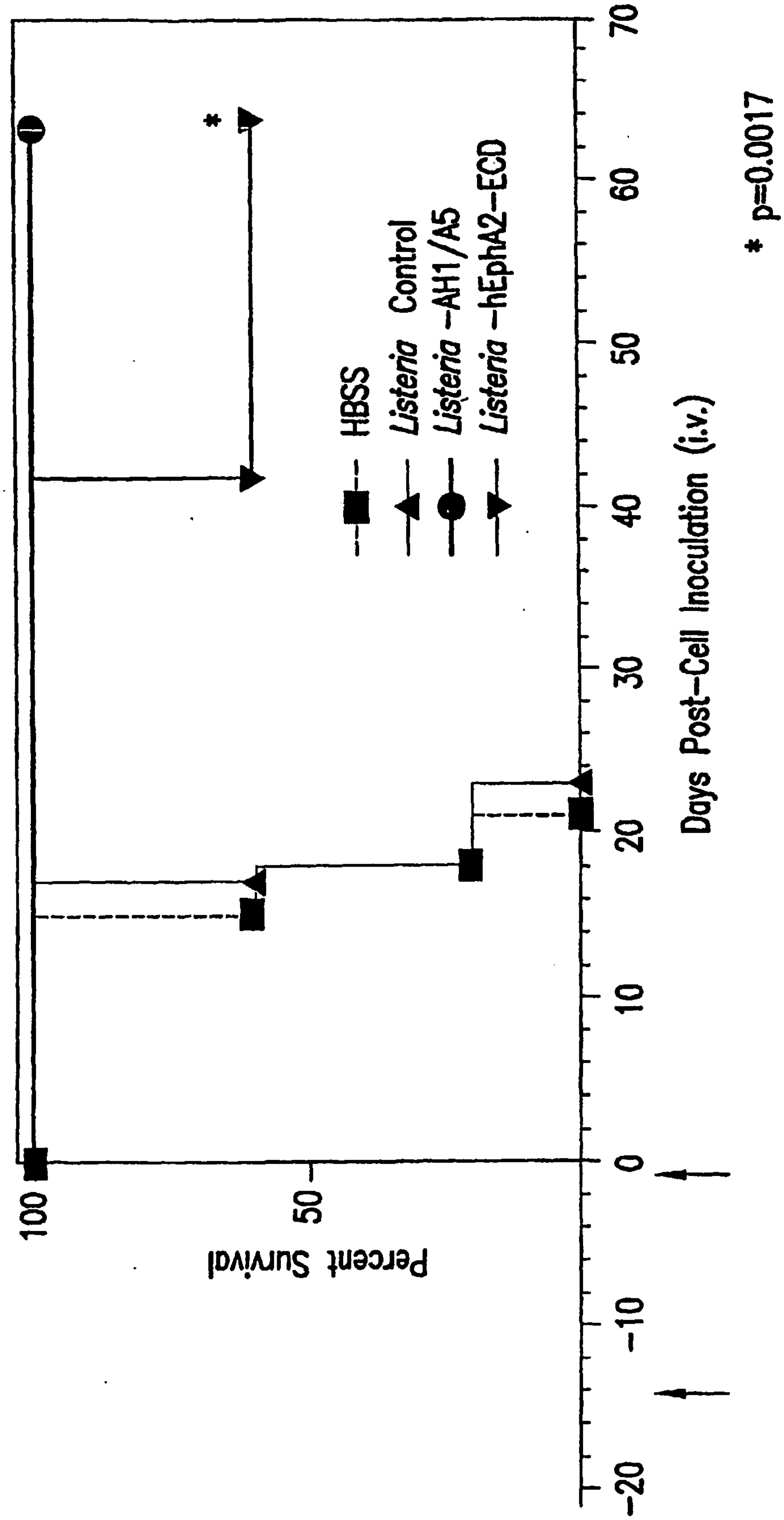

Percent Survival
100
50
Days Post–Cell Inoculation (i.v.)
-20
-10
0
10
20
30
40
50
60
70
HBSS
Listeria Control
Listeria –AH1/A5
Listeria –hEphA2–ECD
*
* p=0.0017

FIG. 10B

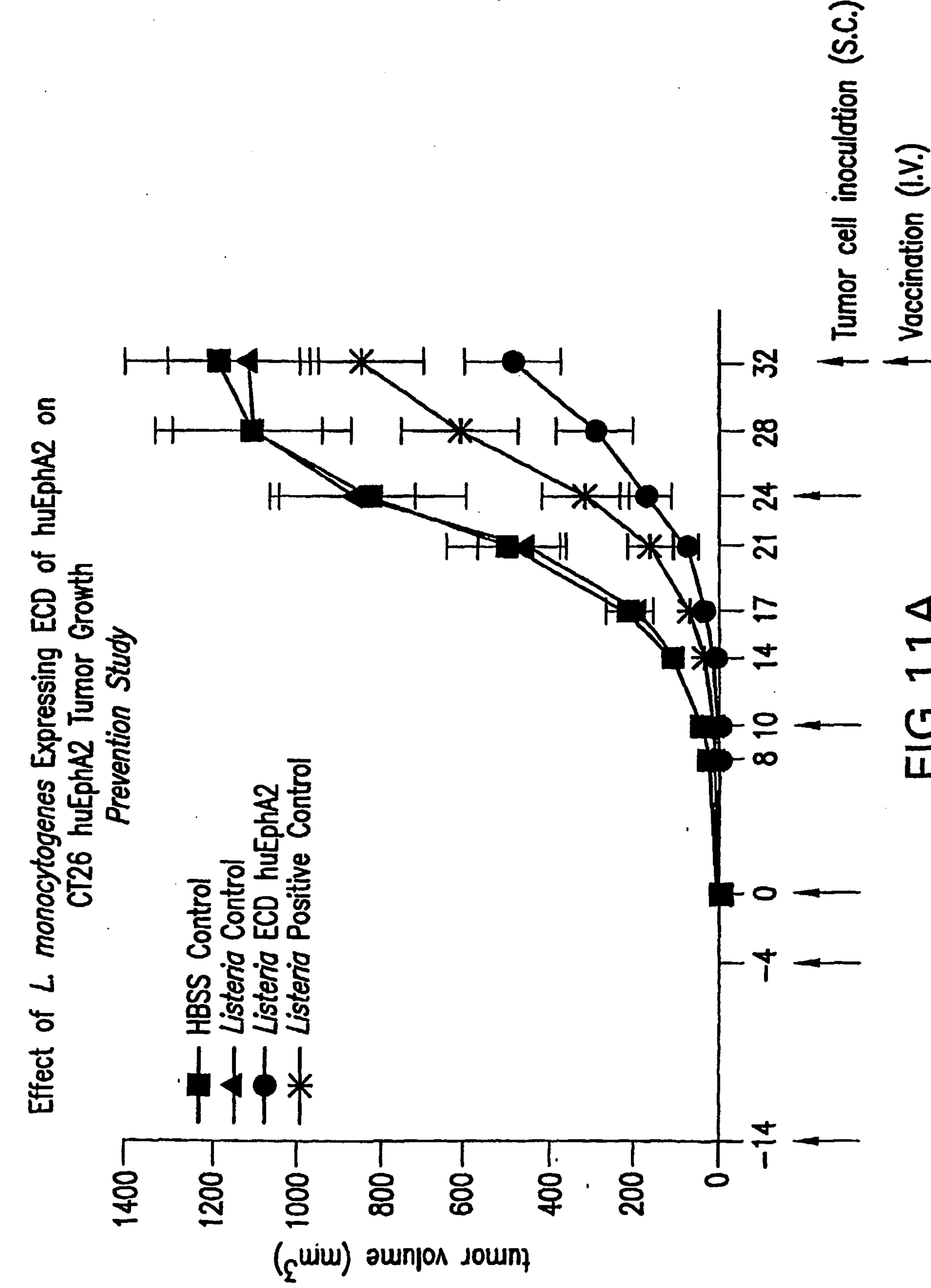

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on CT26 huEphA2 Tumor Growth
Prevention Study
HBSS Control
Listeria Control
Listeria ECD huEphA2
Listeria Positive Control
tumor volume (mm^3)
1400
1200
1000
800
600
400
200
0
−14
−4
0
8
10
14
17
21
24
28
32
Tumor cell inoculation (S.C.)
Vaccination (I.V.)

FIG.11A

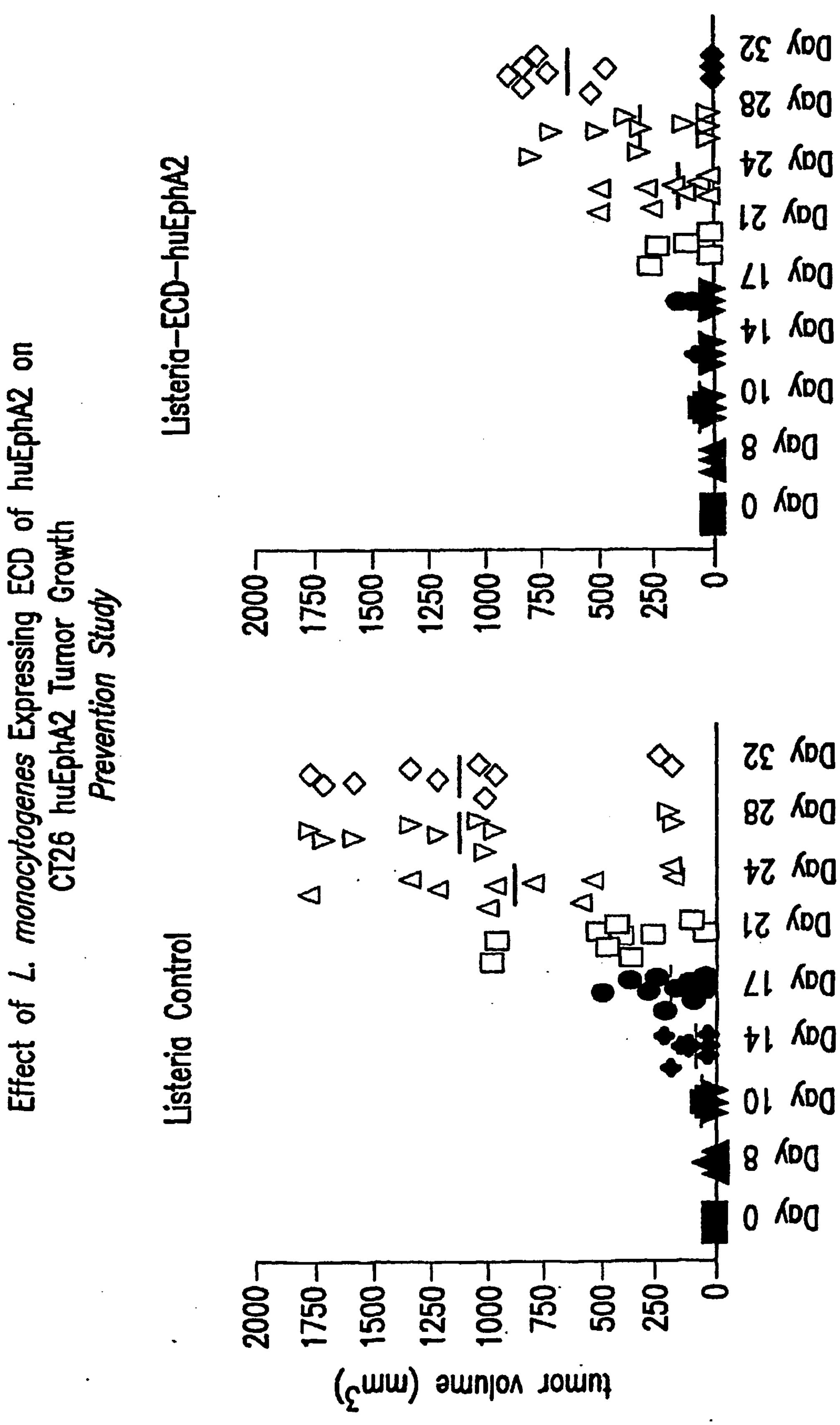

Effect of *L. monocytogenes* Expressing ECD of huEphA2 on
CT26 huEphA2 Tumor Growth
Prevention Study
Listeria Control
Listeria-ECD-huEphA2
tumor volume (mm^3)
2000
1750
1500
1250
1000
750
500
250
0
Day 0
Day 8
Day 10
Day 14
Day 17
Day 21
Day 24
Day 28
Day 32

FIG.11B

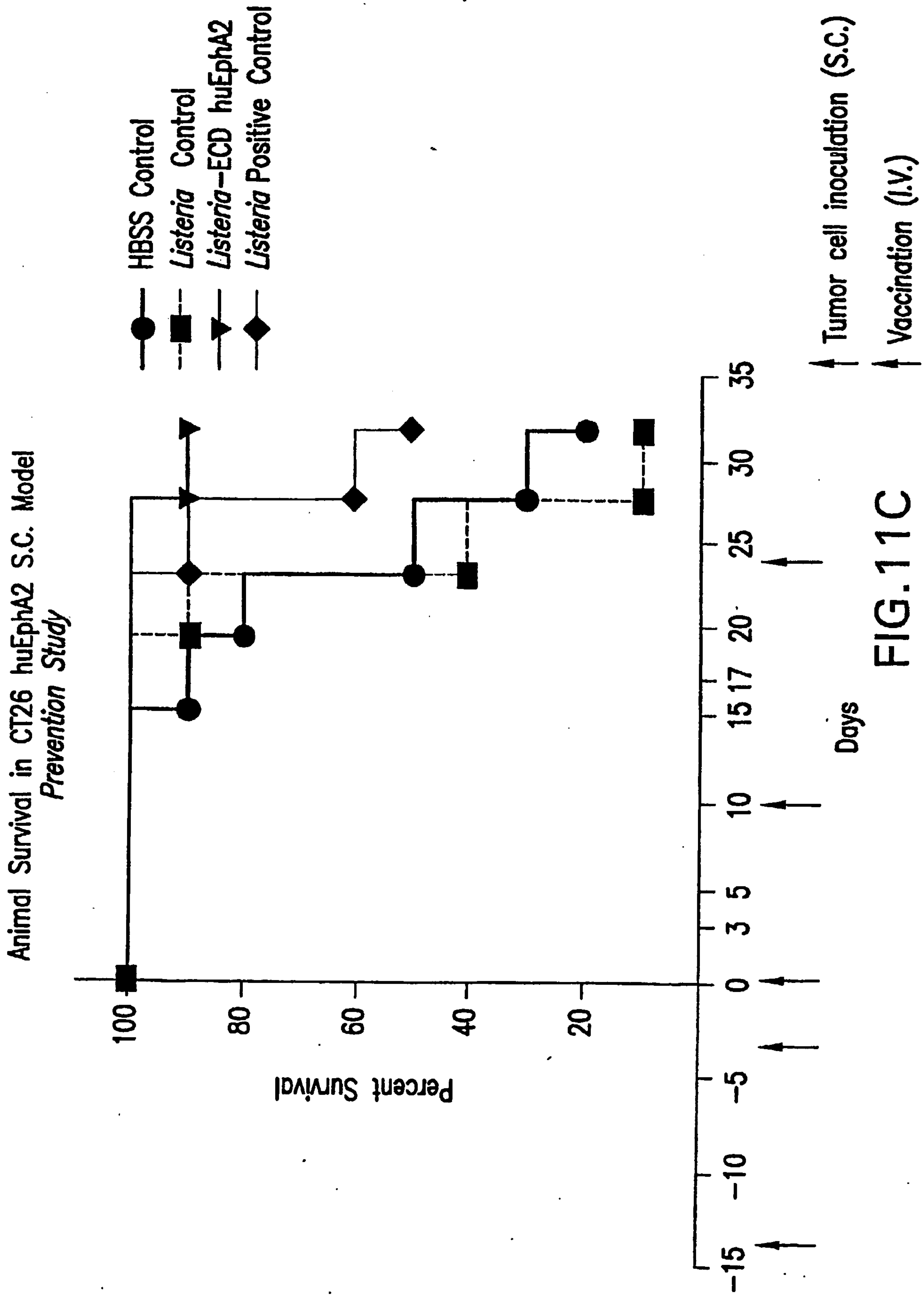

Animal Survival in CT26 huEphA2 S.C. Model
Prevention Study
Percent Survival
100
80
60
40
20
−15
−10
−5
0
3
5
10
15
17
20
25
30
35
Days
HBSS Control
Listeria Control
Listeria–ECD huEphA2
Listeria Positive Control
Tumor cell inoculation (S.C.)
Vaccination (I.V.)

FIG. 11C

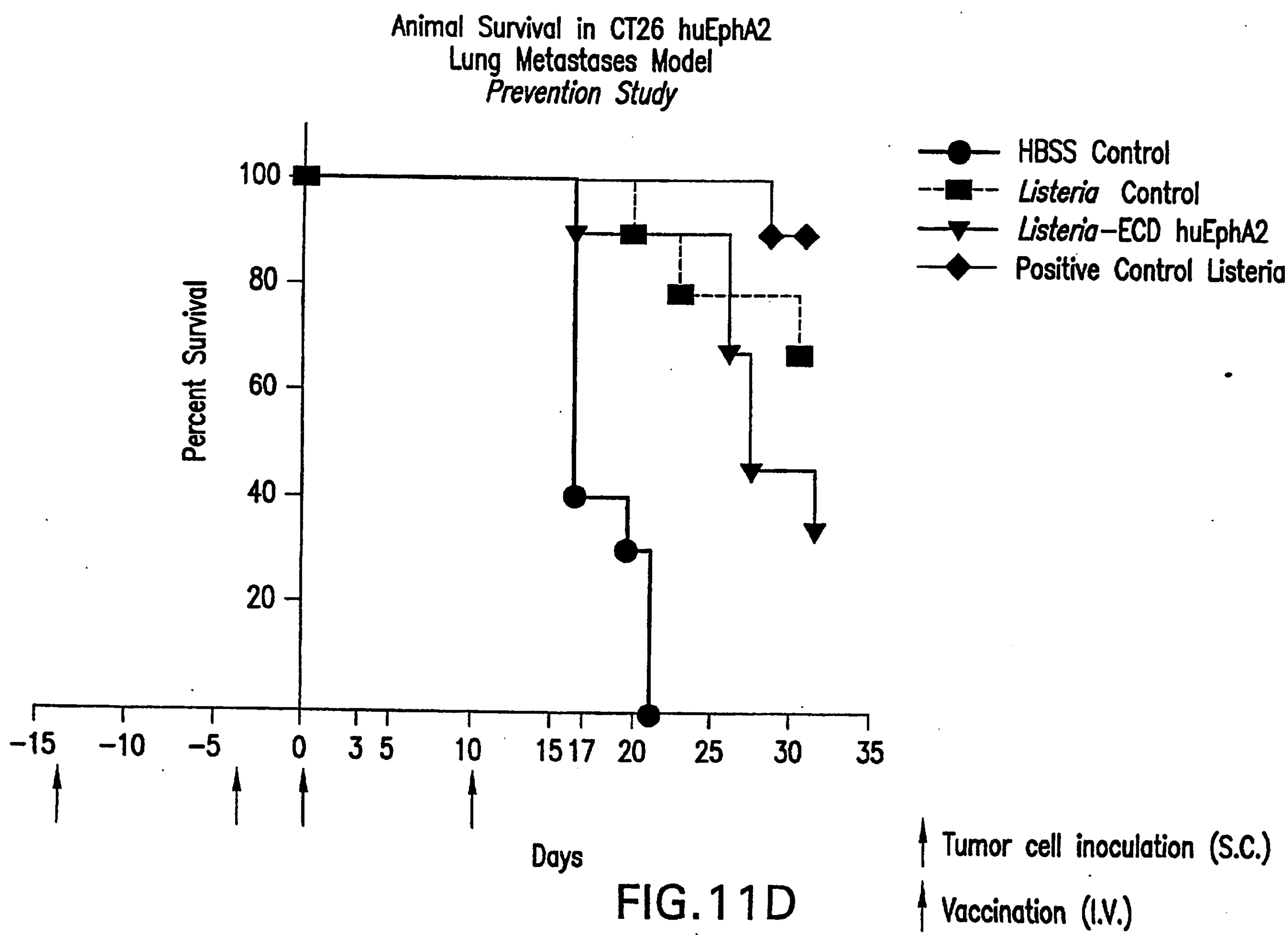
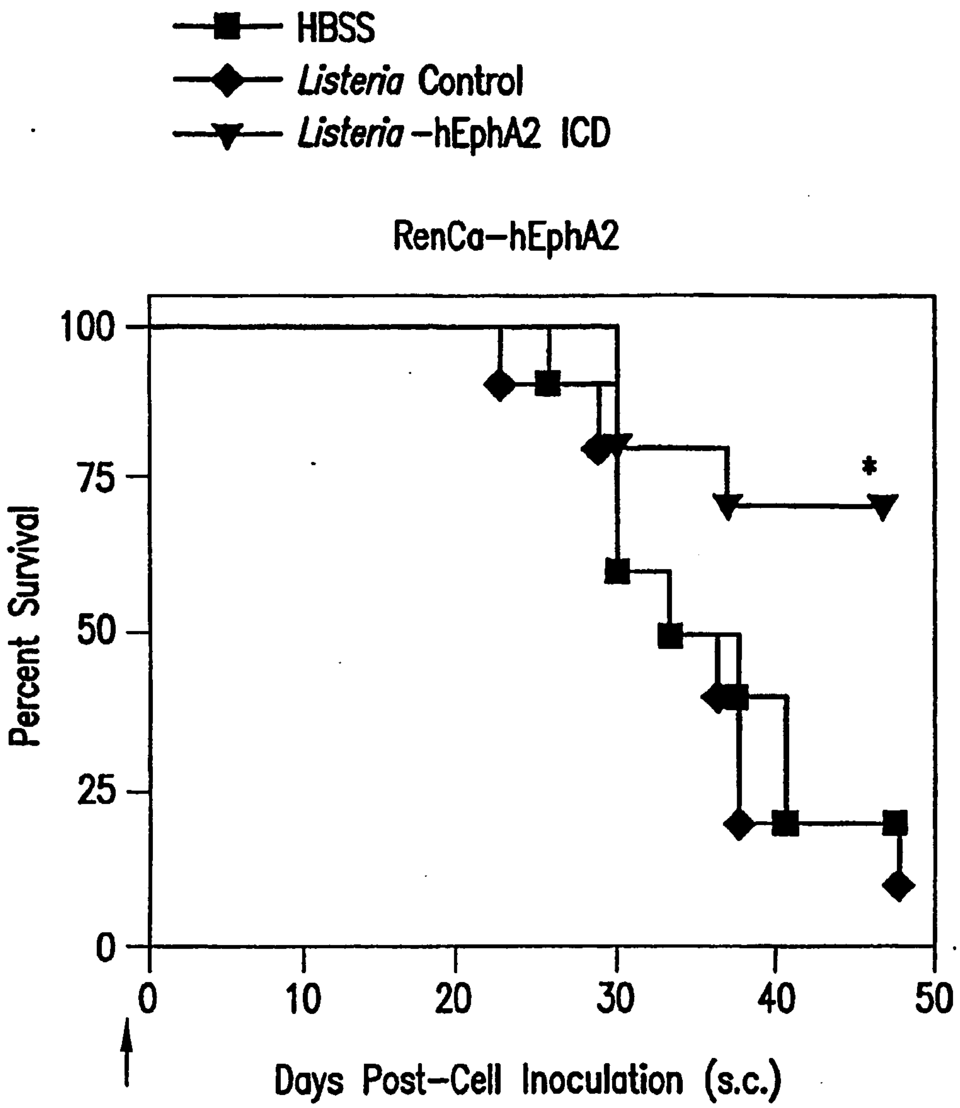

Animal Survival in CT26 huEphA2
Lung Metastases Model
Prevention Study
Percent Survival
100
80
60
40
20
−15
−10
−5
0
3 5
10
15 17
20
25
30
35
Days
HBSS Control
Listeria Control
Listeria−ECD huEphA2
Positive Control Listeria
Tumor cell inoculation (S.C.)
Vaccination (I.V.)

FIG.11D



HBSS
Listeria Control
Listeria-hEphA2 ICD
RenCa-hEphA2
Percent Survival
100
75
50
25
0
0
10
20
30
40
50
*
Days Post-Cell Inoculation (s.c.)

FIG.12

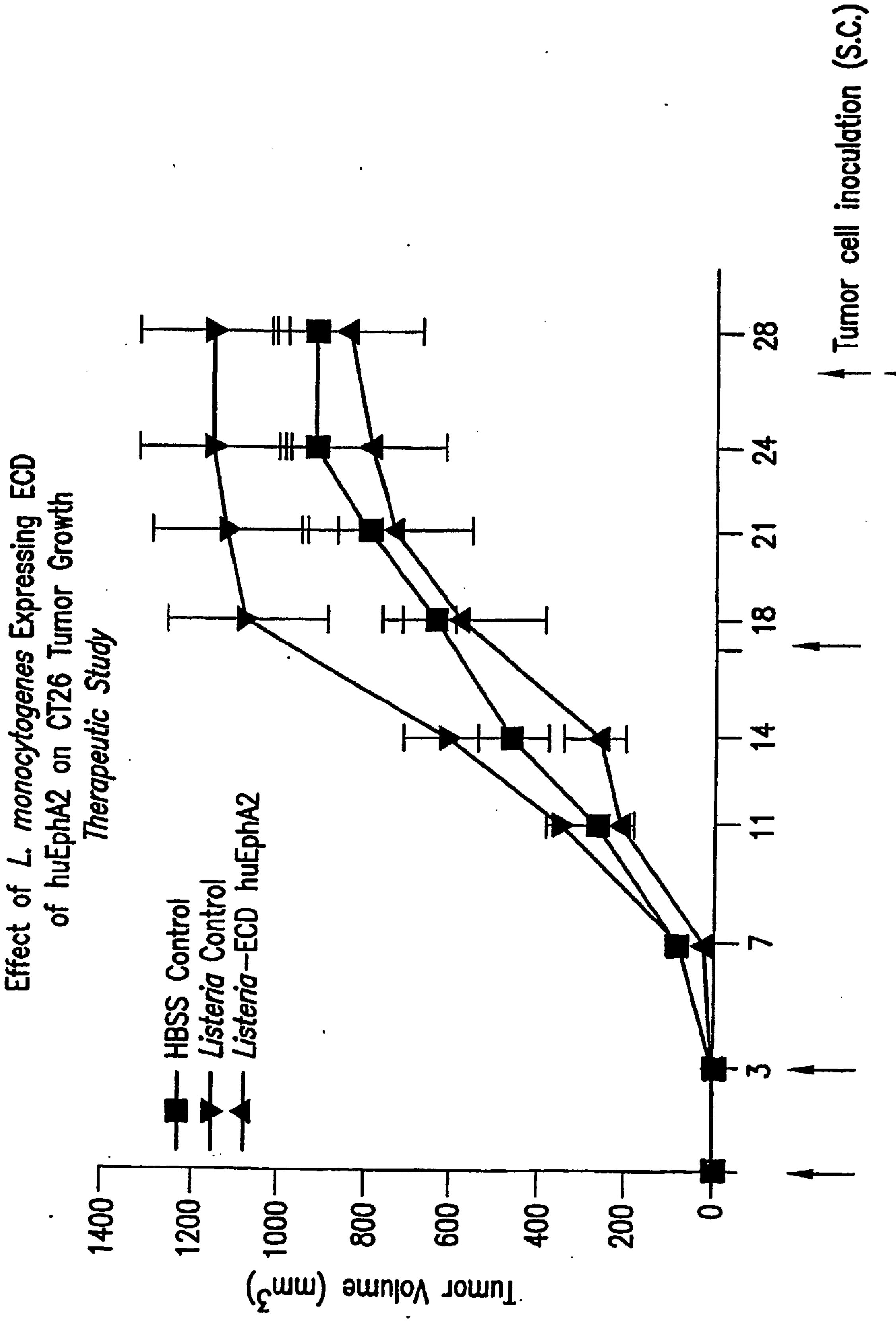

Effect of L. monocytogenes Expressing ECD of huEphA2 on CT26 Tumor Growth
Therapeutic Study
HBSS Control
Listeria Control
Listeria—ECD huEphA2
Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
3
7
11
14
18
21
24
28
Tumor cell inoculation (S.C.)
Vaccination (I.V.)

FIG.13A

18/30

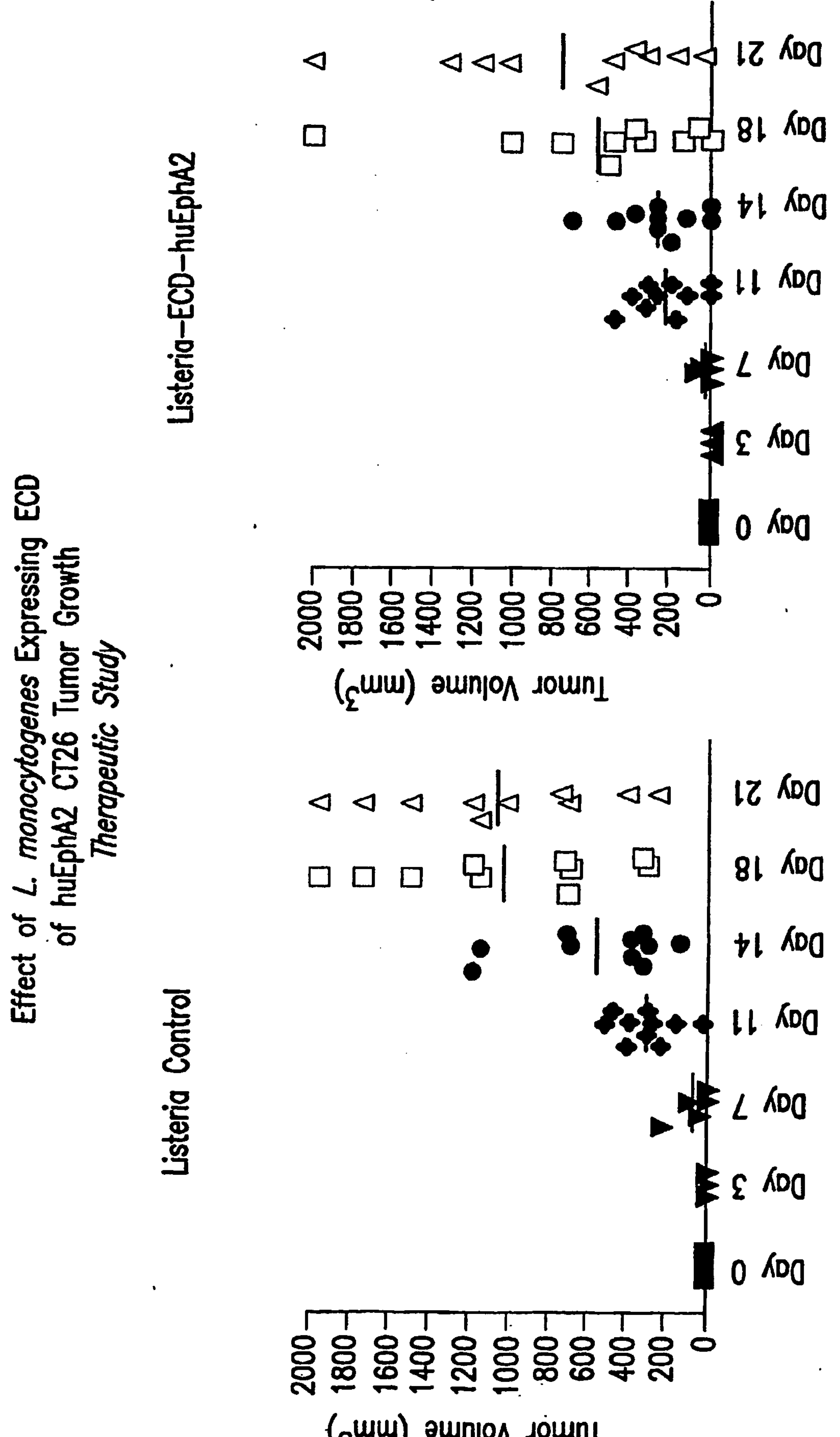
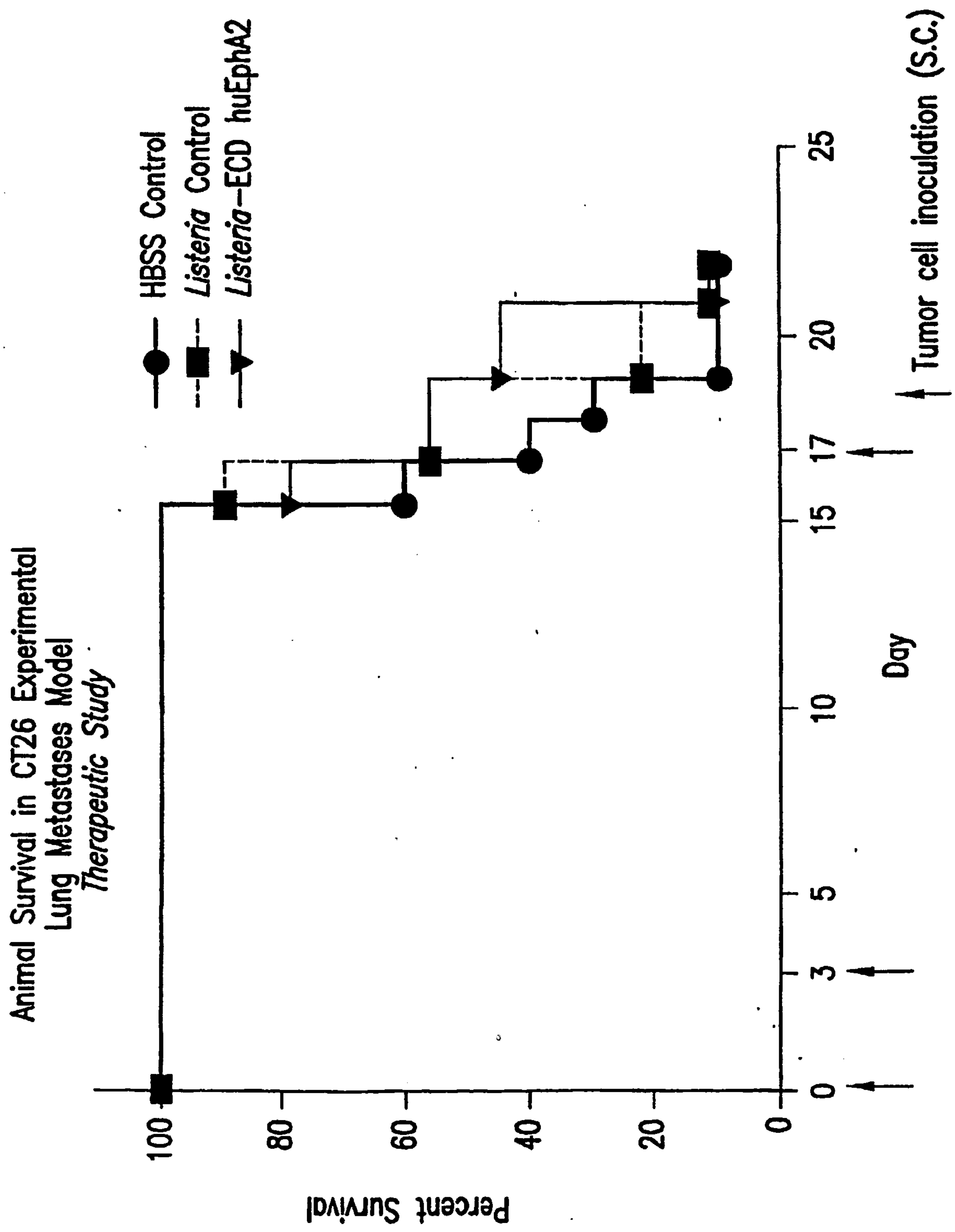

Effect of L. monocytogenes Expressing ECD
of huEphA2 CT26 Tumor Growth
Therapeutic Study
Listeria Control
Listeria-ECD-huEphA2
Tumor Volume (mm^3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Day 0
Day 3
Day 7
Day 11
Day 14
Day 18
Day 21

FIG.13B



Animal Survival in CT26 Experimental
Lung Metastases Model
Therapeutic Study
HBSS Control
Listeria Control
Listeria–ECD huEphA2
Percent Survival
100
80
60
40
20
0
0
3
5
10
15
17
20
25
Day
Tumor cell inoculation (S.C.)
Vaccination (I.V.)

FIG.13C

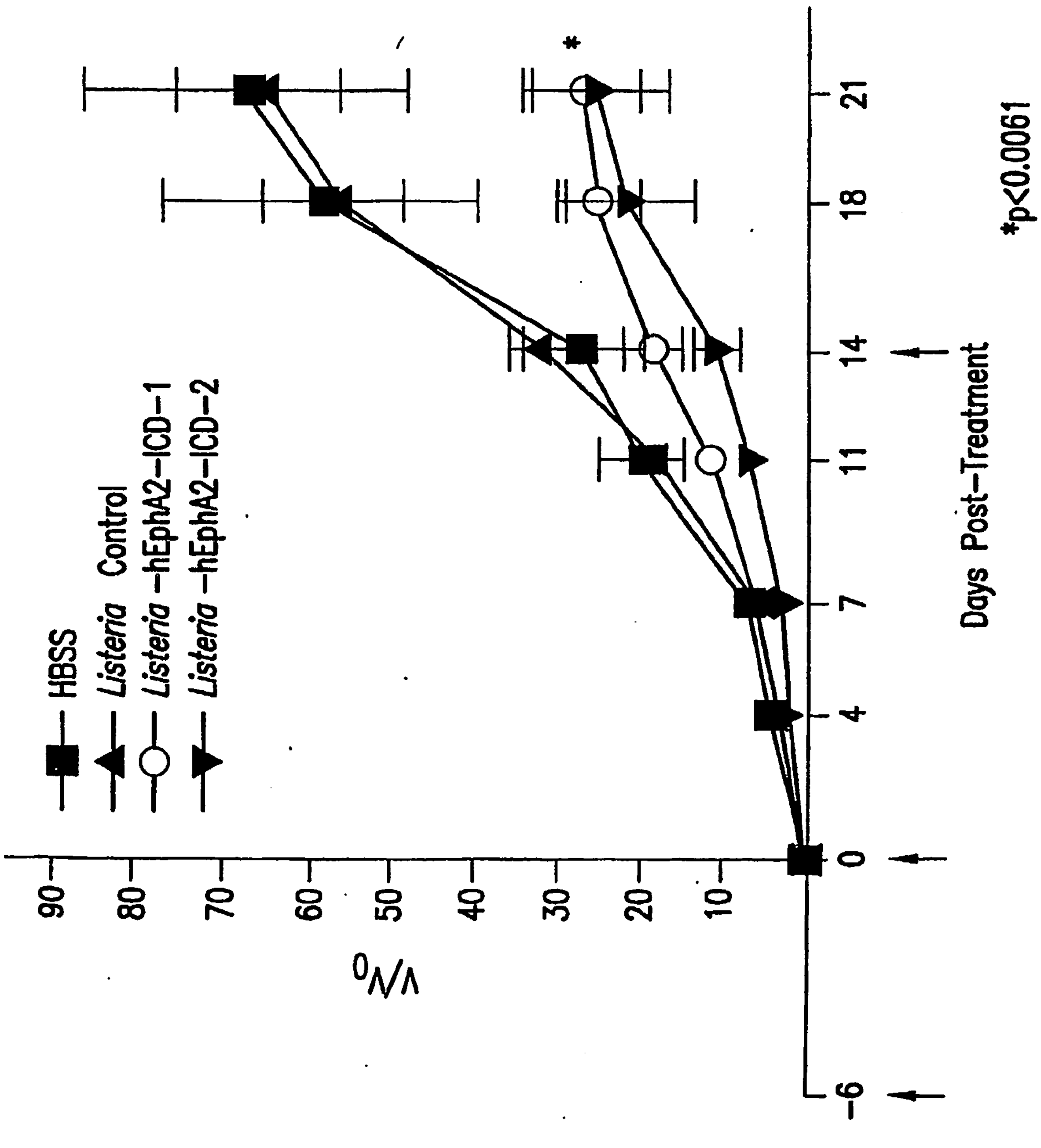

HBSS
Listeria Control
Listeria -hEphA2-ICD-1
Listeria -hEphA2-ICD-2
V/V0
90
80
70
60
50
40
30
20
10
-6
0
4
7
11
14
18
21
*
Days Post-Treatment
*p<0.0061

FIG.14A

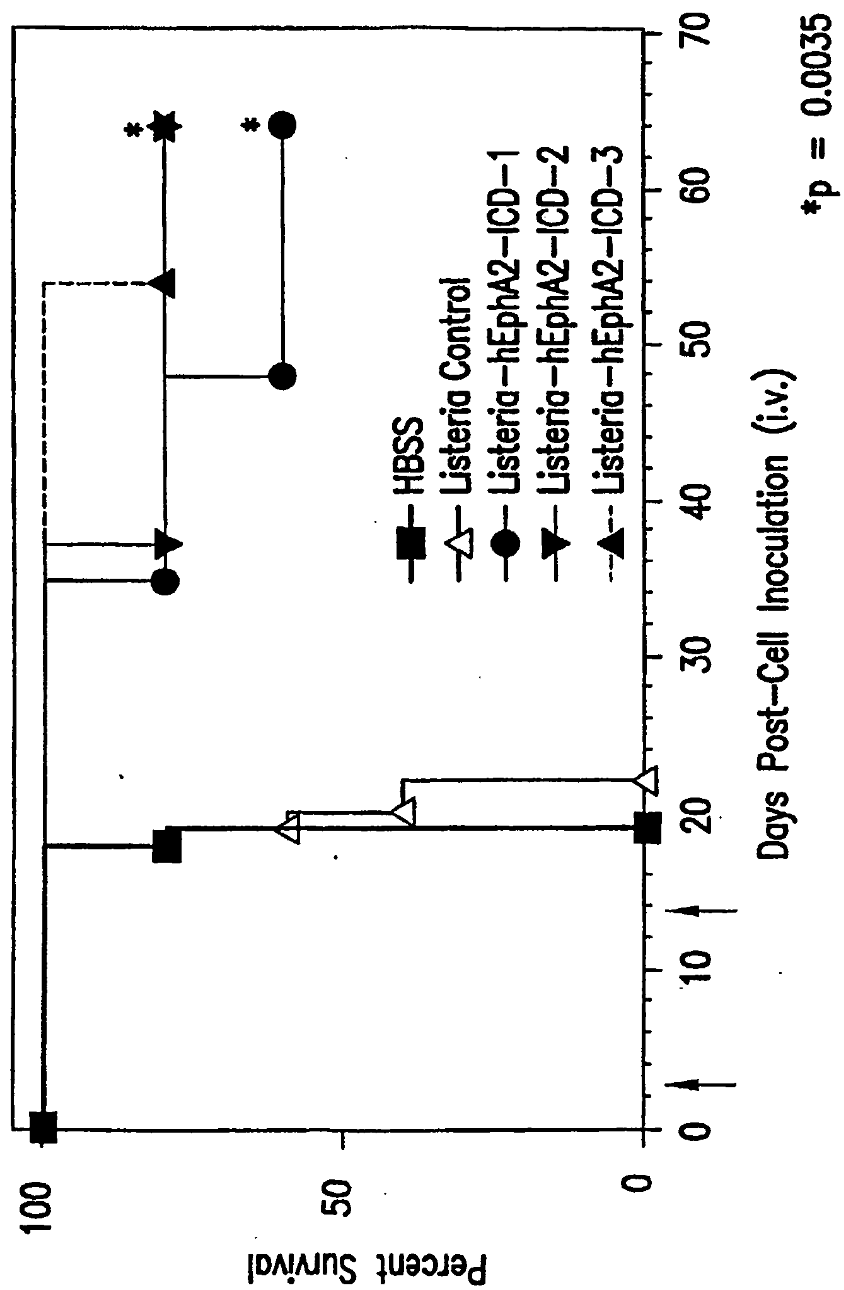
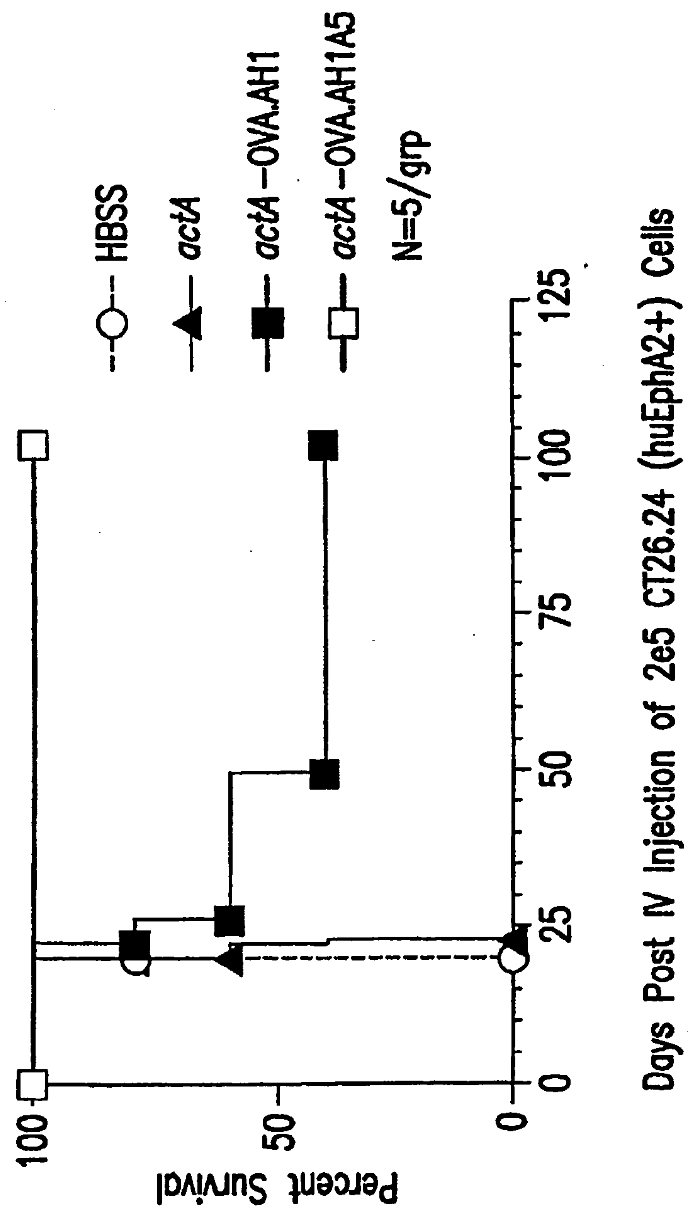

Percent Survival
100
50
0
0
10
20
30
40
50
60
70
Days Post-Cell Inoculation (i.v.)
HBSS
Listeria Control
Listeria-hEphA2-ICD-1
Listeria-hEphA2-ICD-2
Listeria-hEphA2-ICD-3
*
*
*p = 0.0035

FIG.14B



Percent Survival
100
50
0
0
25
50
75
100
125
Days Post IV Injection of 2e5 CT26.24 (huEphA2+) Cells
HBSS
actA
actA —OVA.AH1
actA —OVA.AH1A5
N=5/grp

FIG.14C

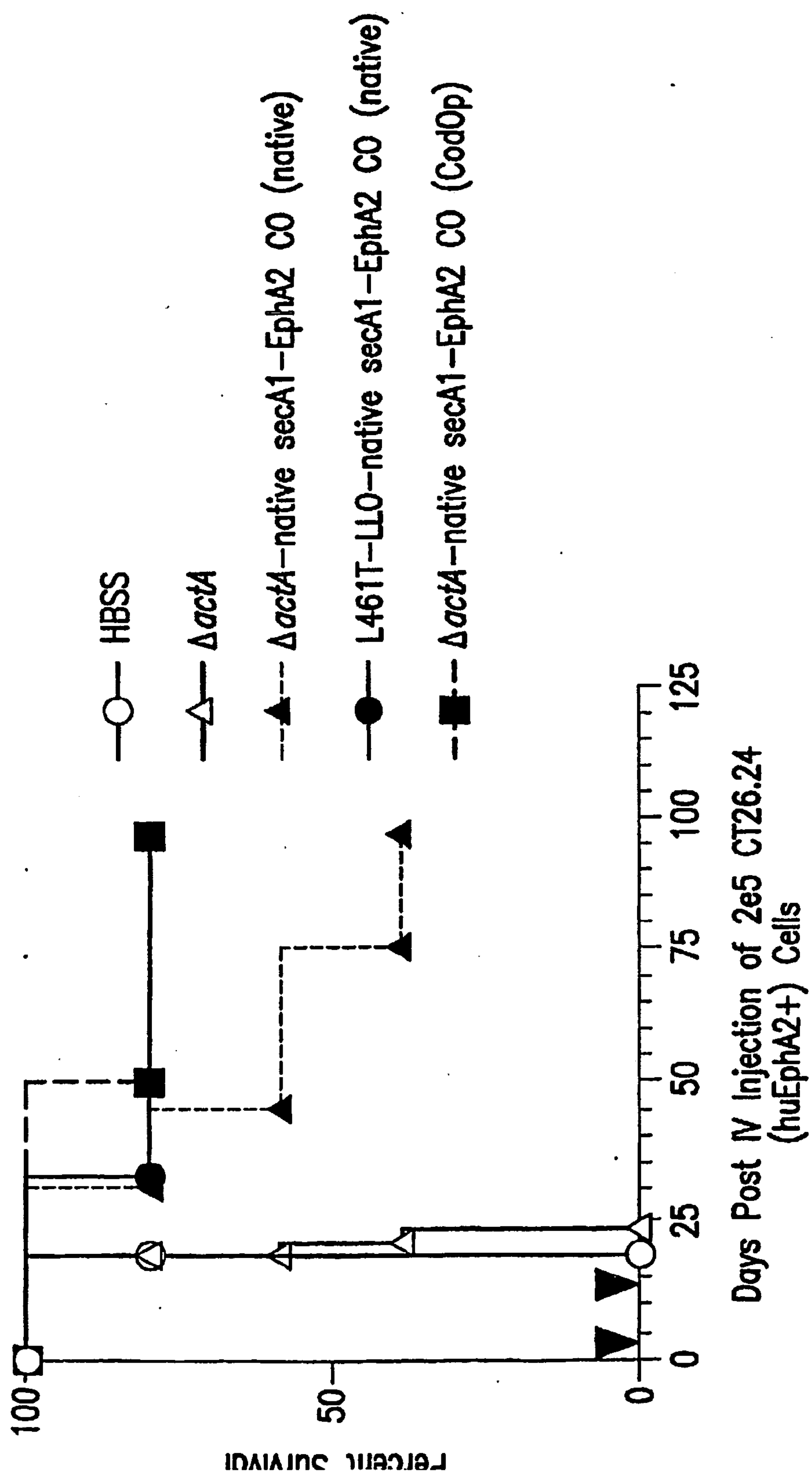

HBSS
ΔactA
ΔactA–native secA1–EphA2 CO (native)
L461T–LLO–native secA1–EphA2 CO (native)
ΔactA–native secA1–EphA2 CO (CodOp)
Percent Survival
100
50
0
0
25
50
75
100
125
Days Post IV Injection of 2e5 CT26.24 (huEphA2+) Cells

FIG.14D

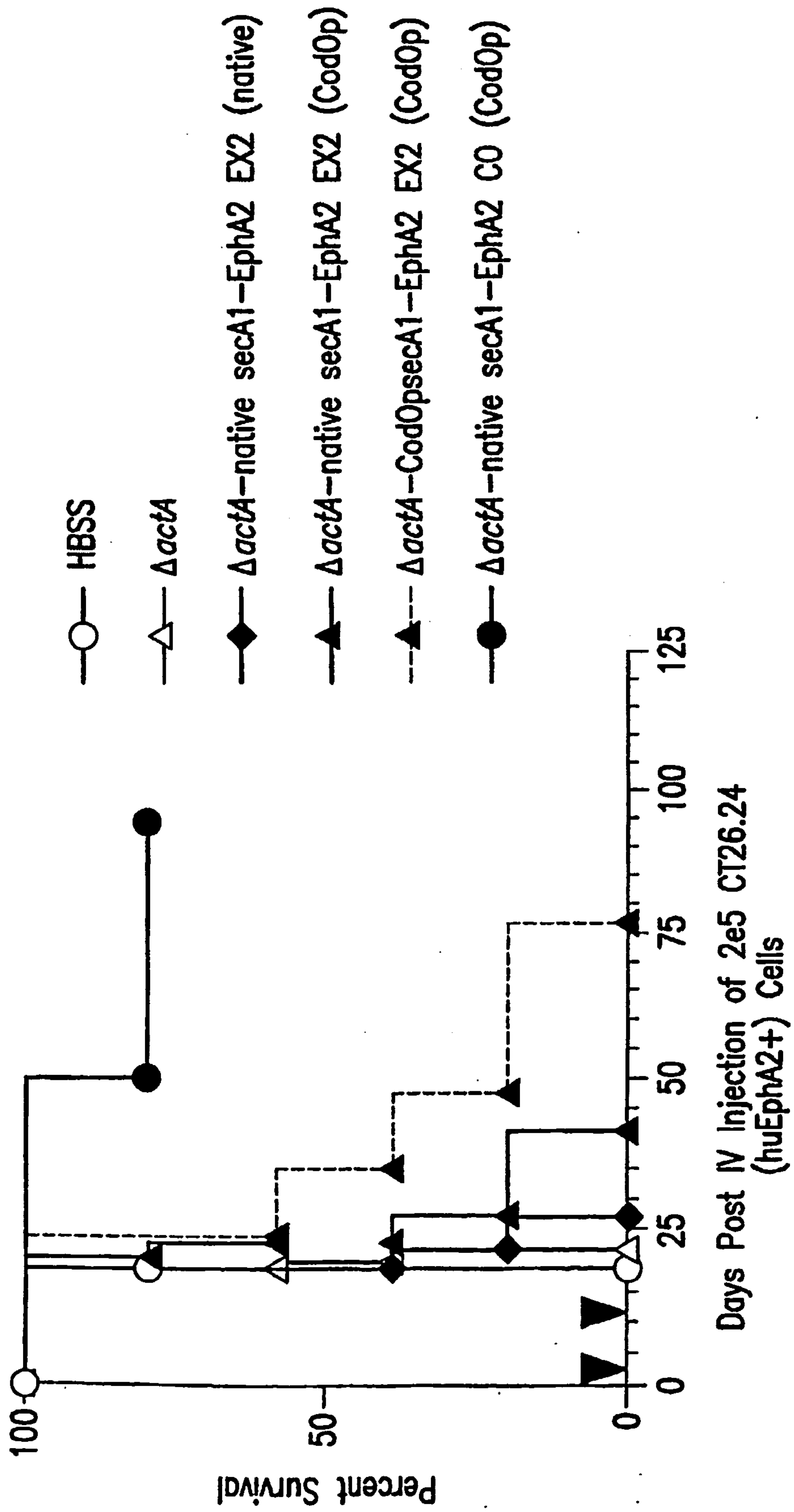

Percent Survival
100
50
0
0
25
50
75
100
125
Days Post IV Injection of 2e5 CT26.24
(huEphA2+) Cells
HBSS
ΔactA
ΔactA–native secA1–EphA2 EX2 (native)
ΔactA–native secA1–EphA2 EX2 (CodOp)
ΔactA–CodOpsecA1–EphA2 EX2 (CodOp)
ΔactA–native secA1–EphA2 CO (CodOp)

FIG.14E

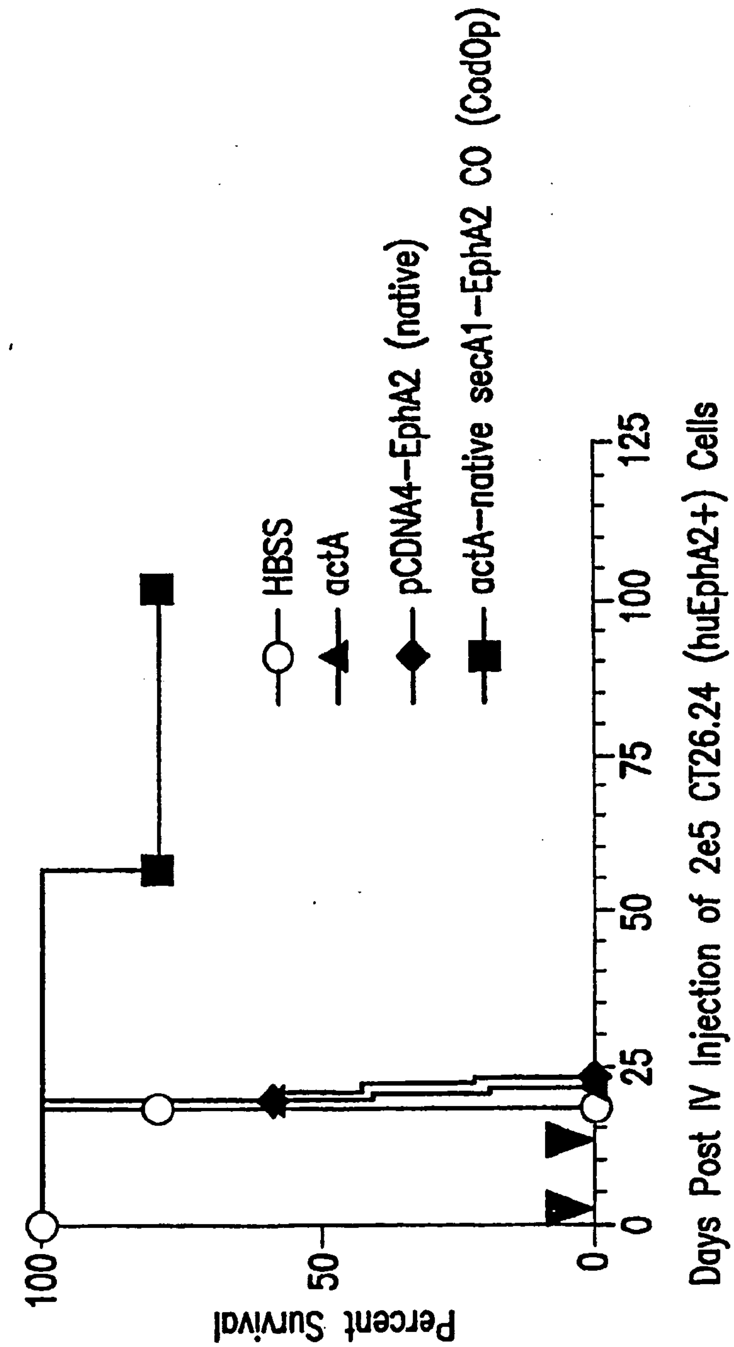

HBSS
actA
pCDNA4–EphA2 (native)
actA–native secA1–EphA2 CO (CodOp)
Percent Survival
100
50
0
0
25
50
75
100
125
Days Post IV Injection of 2e5 CT26.24 (huEphA2+) Cells

FIG.14F

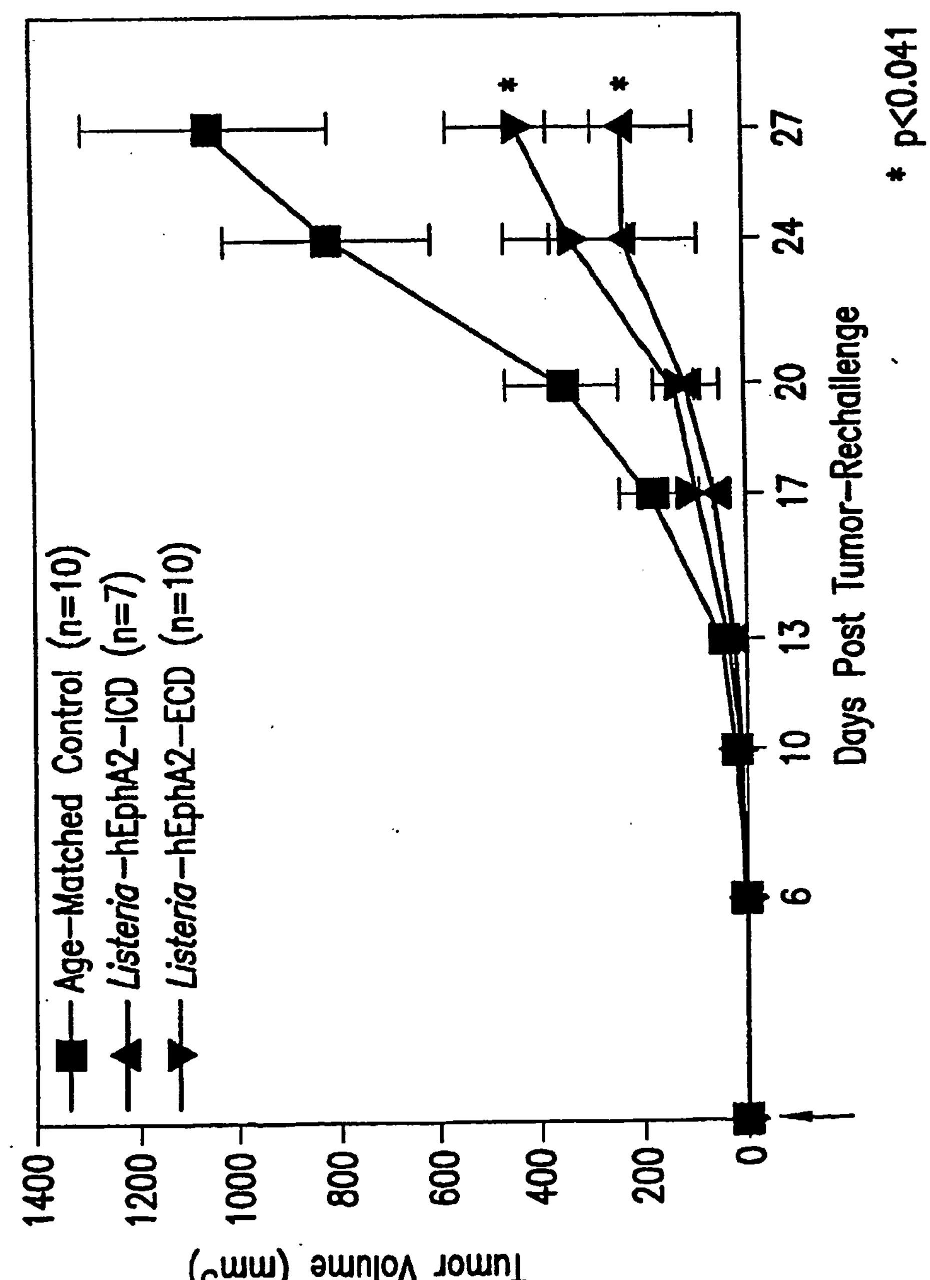

Age-Matched Control (n=10)
Listeria-hEphA2-ICD (n=7)
Listeria-hEphA2-ECD (n=10)
Tumor Volume (mm³)
1400
1200
1000
800
600
400
200
0
0
6
10
13
17
20
24
27
Days Post Tumor-Rechallenge
*
*
* p<0.041

FIG.15

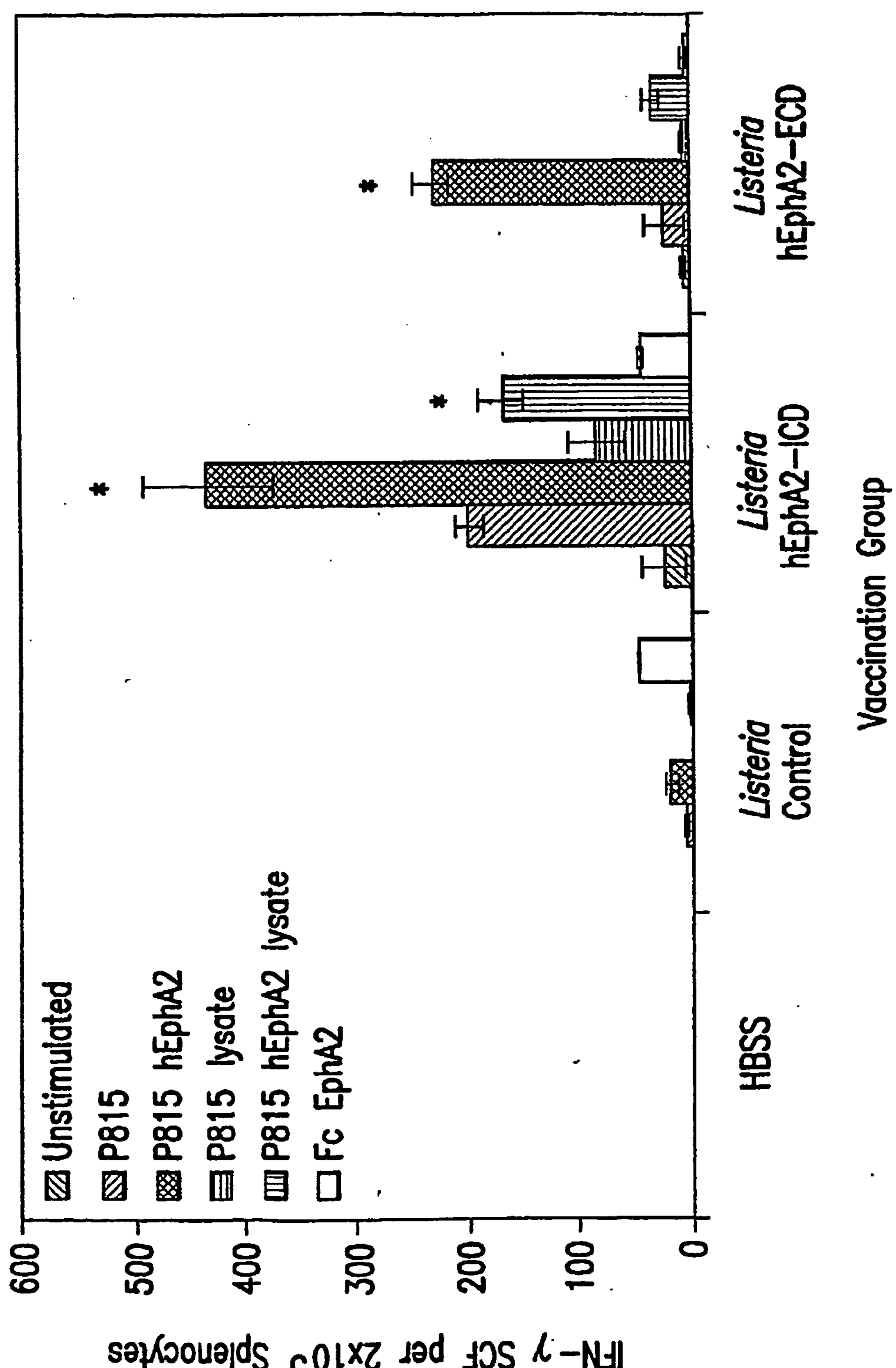

Unstimulated
P815
P815 hEphA2
P815 lysate
P815 hEphA2 lysate
Fc EphA2
IFN-γ SCF per 2x10^5 Splenocytes
600
500
400
300
200
100
0
HBSS
Listeria Control
Listeria hEphA2-ICD
Listeria hEphA2-ECD
Vaccination Group
*
*
*

FIG.16

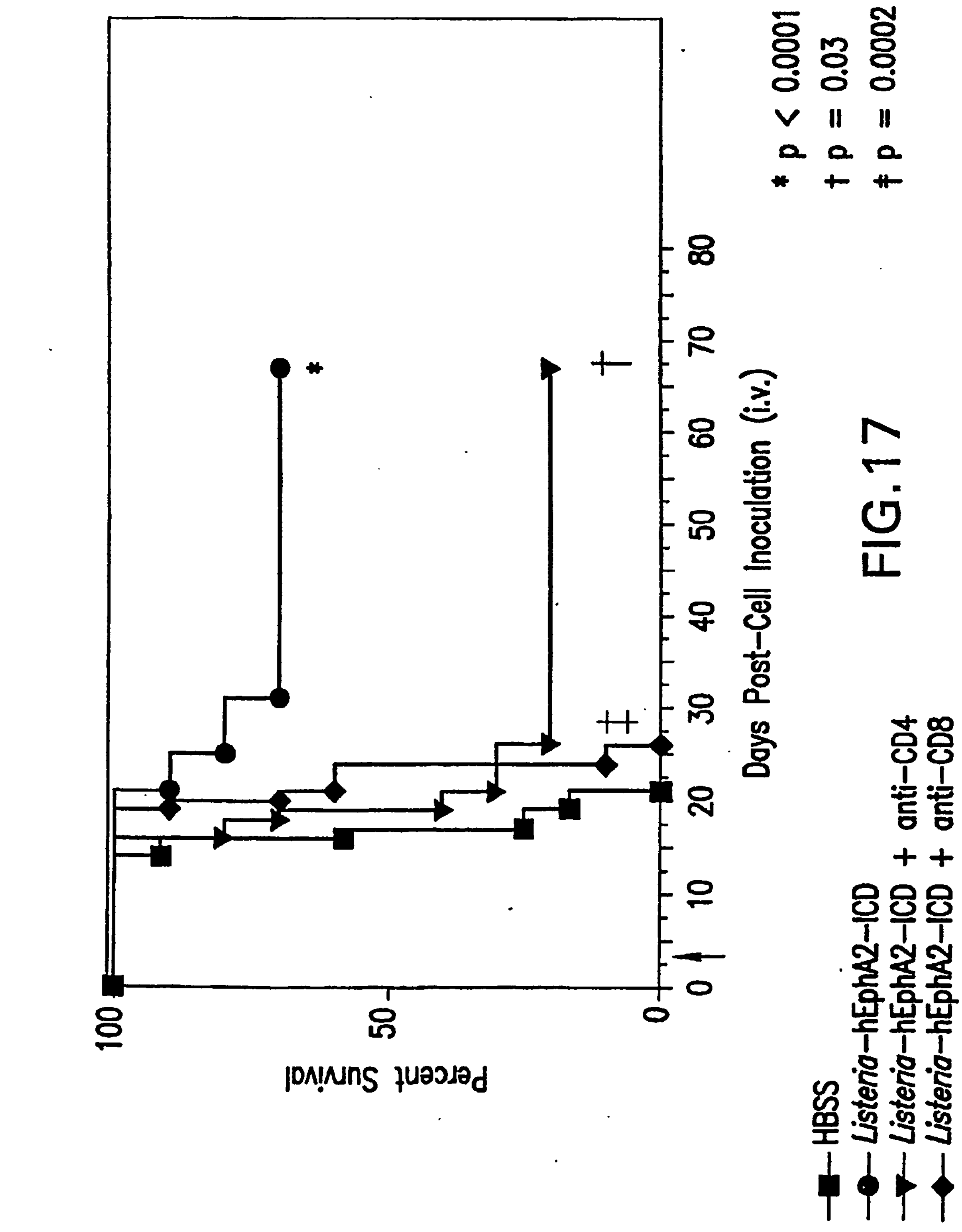

100
50
0
Percent Survival
0
10
20
30
40
50
60
70
80
Days Post–Cell Inoculation (i.v.)
*
†
‡
HBSS
Listeria–hEphA2–ICD
Listeria–hEphA2–ICD + anti–CD4
Listeria–hEphA2–ICD + anti–CD8
* p < 0.0001
† p = 0.03
‡ p = 0.0002

FIG. 17

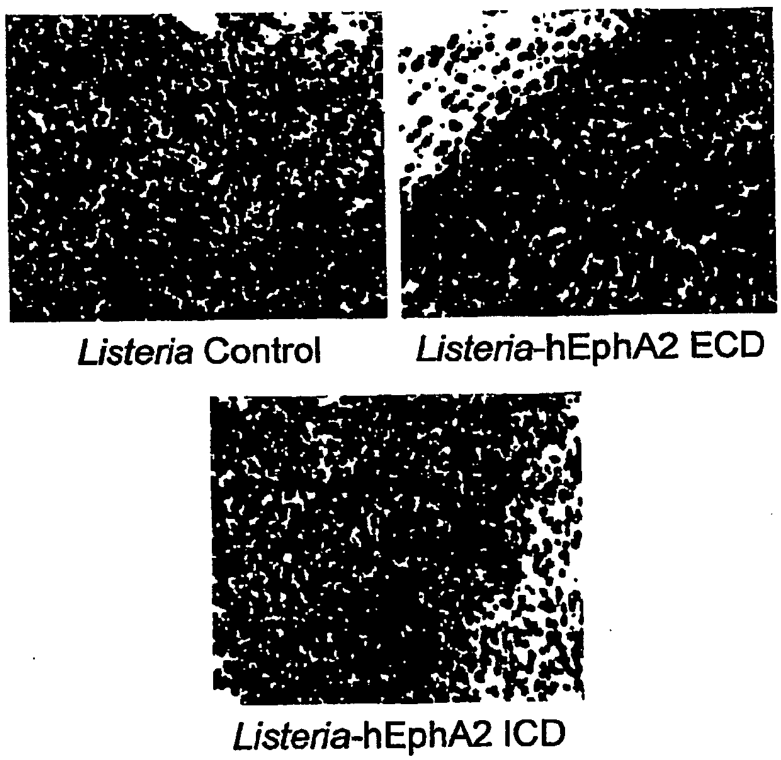


FIG.18A

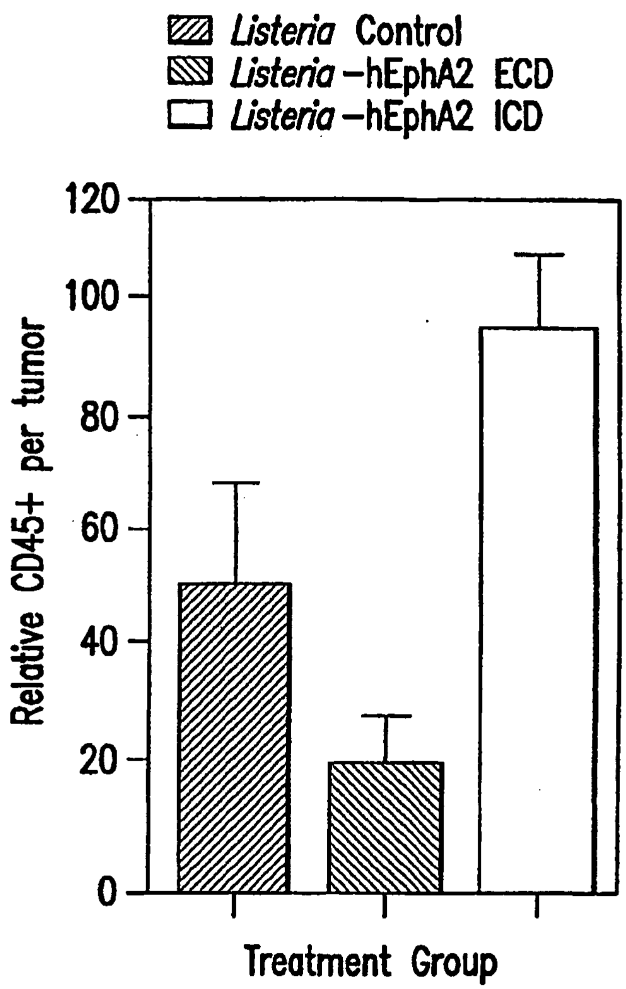

Listeria Control
Listeria –hEphA2 ECD
Listeria –hEphA2 ICD
120
100
80
60
40
20
0
Relative CD45+ per tumor
Treatment Group

FIG.18B

SEQUENCE LISTING

```
<110>  Kinch, Michael S.
       Kiener, Peter A.
       Bruckheimer, Elizabeth
       Dubensky, Jr. Thomas W.
       Cook, David N.

<120>  LISTERIA-BASED EphA2 VACCINES

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<151>  2003-10-15

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agagcgagaa gcgcggc atg gag ctc cag gca gcc cgc gcc tgc ttc gcc        170
                   Met Glu Leu Gln Ala Ala Arg Ala Cys Phe Ala
                   1               5                   10

ctg ctg tgg ggc tgt gcg ctg gcc gcg gcc gcg gcg gcg cag ggc aag       218
Leu Leu Trp Gly Cys Ala Leu Ala Ala Ala Ala Ala Ala Gln Gly Lys
            15                  20                  25

gaa gtg gta ctg ctg gac ttt gct gca gct gga ggg gag ctc ggc tgg       266
Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly Trp
        30                  35                  40

ctc aca cac ccg tat ggc aaa ggg tgg gac ctg atg cag aac atc atg       314
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Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met
45 50 55

aat gac atg ccg atc tac atg tac tcc gtg tgc aac gtg atg tct ggc 362
Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly
60 65 70 75

gac cag gac aac tgg ctc cgc acc aac tgg gtg tac cga gga gag gct 410
Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala
80 85 90

gag cgt atc ttc att gag ctc aag ttt act gta cgt gac tgc aac agc 458
Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser
95 100 105

ttc cct ggt ggc gcc agc tcc tgc aag gag act ttc aac ctc tac tat 506
Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr
110 115 120

gcc gag tcg gac ctg gac tac ggc acc aac ttc cag aag cgc ctg ttc 554
Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe
125 130 135

acc aag att gac acc att gcg ccc gat gag atc acc gtc agc agc gac 602
Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp
140 145 150 155

ttc gag gca cgc cac gtg aag ctg aac gtg gag gag cgc tcc gtg ggg 650
Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly
160 165 170

ccg ctc acc cgc aaa ggc ttc tac ctg gcc ttc cag gat atc ggt gcc 698
Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala
175 180 185

tgt gtg gcg ctg ctc tcc gtc cgt gtc tac tac aag aag tgc ccc gag 746
Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu
190 195 200

ctg ctg cag ggc ctg gcc cac ttc cct gag acc atc gcc ggc tct gat 794
Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp
205 210 215

gca cct tcc ctg gcc act gtg gcc ggc acc tgt gtg gac cat gcc gtg 842
Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp His Ala Val
220 225 230 235

gtg cca ccg ggg ggt gaa gag ccc cgt atg cac tgt gca gtg gat ggc 890
Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp Gly
240 245 250

gag tgg ctg gtg ccc att ggg cag tgc ctg tgc cag gca ggc tac gag 938
Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu
255 260 265

aag gtg gag gat gcc tgc cag gcc tgc tcg cct gga ttt ttt aag ttt 986
Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe
270 275 280

gag gca tct gag agc ccc tgc ttg gag tgc cct gag cac acg ctg cca 1034
Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro

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        285                     290                     295

tcc cct gag ggt gcc acc tcc tgc gag tgt gag gaa ggc ttc ttc cgg      1082
Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg
300                 305                 310                 315

gca cct cag gac cca gcg tcg atg cct tgc aca cga ccc ccc tcc gcc      1130
Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala
                320                 325                 330

cca cac tac ctc aca gcc gtg ggc atg ggt gcc aag gtg gag ctg cgc      1178
Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg
            335                 340                 345

tgg acg ccc cct cag gac agc ggg ggc cgc gag gac att gtc tac agc      1226
Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser
        350                 355                 360

gtc acc tgc gaa cag tgc tgg ccc gag tct ggg gaa tgc ggg ccg tgt      1274
Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys
    365                 370                 375

gag gcc agt gtg cgc tac tcg gag cct cct cac gga ctg acc cgc acc      1322
Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr
380                 385                 390                 395

agt gtg aca gtg agc gac ctg gag ccc cac atg aac tac acc ttc acc      1370
Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr
                400                 405                 410

gtg gag gcc cgc aat ggc gtc tca ggc ctg gta acc agc cgc agc ttc      1418
Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe
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cgt act gcc agt gtc agc atc aac cag aca gag ccc ccc aag gtg agg      1466
Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg
        430                 435                 440

ctg gag ggc cgc agc acc acc tcg ctt agc gtc tcc tgg agc atc ccc      1514
Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro
    445                 450                 455

ccg ccg cag cag agc cga gtg tgg aag tac gag gtc act tac cgc aag      1562
Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys
460                 465                 470                 475

aag gga gac tcc aac agc tac aat gtg cgc cgc acc gag ggt ttc tcc      1610
Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser
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gtg acc ctg gac gac ctg gcc cca gac acc acc tac ctg gtc cag gtg      1658
Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val
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cag gca ctg acg cag gag ggc cag ggg gcc ggc agc aag gtg cac gaa      1706
Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Lys Val His Glu
        510                 515                 520

ttc cag acg ctg tcc ccg gag gga tct ggc aac ttg gcg gtg att ggc      1754
Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly Asn Leu Ala Val Ile Gly
    525                 530                 535
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ggc gtg gct gtc ggt gtg gtc ctg ctt ctg gtg ctg gca gga gtt ggc      1802
Gly Val Ala Val Gly Val Val Leu Leu Leu Val Leu Ala Gly Val Gly
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ttc ttt atc cac cgc agg agg aag aac cag cgt gcc cgc cag tcc ccg      1850
Phe Phe Ile His Arg Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro
                560                 565                 570

gag gac gtt tac ttc tcc aag tca gaa caa ctg aag ccc ctg aag aca      1898
Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr
            575                 580                 585

tac gtg gac ccc cac aca tat gag gac ccc aac cag gct gtg ttg aag      1946
Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys
        590                 595                 600

ttc act acc gag atc cat cca tcc tgt gtc act cgg cag aag gtg atc      1994
Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln Lys Val Ile
    605                 610                 615

gga gca gga gag ttt ggg gag gtg tac aag ggc atg ctg aag aca tcc      2042
Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser
620                 625                 630                 635

tcg ggg aag aag gag gtg ccg gtg gcc atc aag acg ctg aaa gcc ggc      2090
Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly
                640                 645                 650

tac aca gag aag cag cga gtg gac ttc ctc ggc gag gcc ggc atc atg      2138
Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met
            655                 660                 665

ggc cag ttc agc cac cac aac atc atc cgc cta gag ggc gtc atc tcc      2186
Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser
        670                 675                 680

aaa tac aag ccc atg atg atc atc act gag tac atg gag aat ggg gcc      2234
Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala
    685                 690                 695

ctg gac aag ttc ctt cgg gag aag gat ggc gag ttc agc gtg ctg cag      2282
Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln
700                 705                 710                 715

ctg gtg ggc atg ctg cgg ggc atc gca gct ggc atg aag tac ctg gcc      2330
Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala
                720                 725                 730

aac atg aac tat gtg cac cgt gac ctg gct gcc cgc aac atc ctc gtc      2378
Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val
            735                 740                 745

aac agc aac ctg gtc tgc aag gtg tct gac ttt ggc ctg tcc cgc gtg      2426
Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val
        750                 755                 760

ctg gag gac gac ccc gag gcc acc tac acc acc agt ggc ggc aag atc      2474
Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile
    765                 770                 775
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ccc atc cgc tgg acc gcc ccg gag gcc att tcc tac cgg aag ttc acc      2522
Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr
780                 785                 790                 795

tct gcc agc gac gtg tgg agc ttt ggc att gtc atg tgg gag gtg atg      2570
Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu Val Met
                800                 805                 810

acc tat ggc gag cgg ccc tac tgg gag ttg tcc aac cac gag gtg atg      2618
Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met
            815                 820                 825

aaa gcc atc aat gat ggc ttc cgg ctc ccc aca ccc atg gac tgc ccc      2666
Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro
        830                 835                 840

tcc gcc atc tac cag ctc atg atg cag tgc tgg cag cag gag cgt gcc      2714
Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala
    845                 850                 855

cgc cgc ccc aag ttc gct gac atc gtc agc atc ctg gac aag ctc att      2762
Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile
860                 865                 870                 875

cgt gcc cct gac tcc ctc aag acc ctg gct gac ttt gac ccc cgc gtg      2810
Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val
                880                 885                 890

tct atc cgg ctc ccc agc acg agc ggc tcg gag ggg gtg ccc ttc cgc      2858
Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg
            895                 900                 905

acg gtg tcc gag tgg ctg gag tcc atc aag atg cag cag tat acg gag      2906
Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu
        910                 915                 920

cac ttc atg gcg gcc ggc tac act gcc atc gag aag gtg gtg cag atg      2954
His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met
    925                 930                 935

acc aac gac gac atc aag agg att ggg gtg cgg ctg ccc ggc cac cag      3002
Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln
940                 945                 950                 955

aag cgc atc gcc tac agc ctg ctg gga ctc aag gac cag gtg aac act      3050
Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr
                960                 965                 970

gtg ggg atc ccc atc tga gcctcgacag ggcctggagc cccatcggcc            3098
Val Gly Ile Pro Ile
            975

aagaatactt gaagaaacag agtggcctcc ctgctgtgcc atgctgggcc actggggact   3158

ttatttattt ctagttcttt cctcccctg caacttccgc tgaggggtct cggatgacac    3218

cctggcctga actgaggaga tgaccaggga tgctgggctg ggccctcttt ccctgcgaga   3278

cgcacacagc tgagcactta gcaggcaccg ccacgtccca gcatccctgg agcaggagcc   3338

ccgccacagc cttcggacag acatatagga tattcccaag ccgaccttcc ctccgccttc   3398
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tcccacatga ggccatctca ggagatggag ggcttggccc agcgccaagt aaacagggta 3458

cctcaagccc catttcctca cactaagagg gcagactgtg aacttgactg ggtgagaccc 3518

aaagcggtcc ctgtccctct agtgccttct ttagaccctc gggccccatc ctcatccctg 3578

actggccaaa cccttgcttt cctgggcctt tgcaagatgc ttggttgtgt tgaggttttt 3638

aaatatatat tttgtacttt gtggagagaa tgtgtgtgtg tggcaggggg ccccgccagg 3698

gctggggaca gagggtgtca aacattcgtg agctggggac tcagggaccg gtgctgcagg 3758

agtgtcctgc ccatgcccca gtcggcccca tctctcatcc ttttggataa gtttctattc 3818

tgtcagtgtt aaagattttg ttttgttgga catttttttc gaatcttaat ttattatttt 3878

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caaaccgaaa aaaaaaaaaa aaaaa 3963

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Ala Leu Ala Ala Ala Ala Ala Ala Gln Gly Lys Glu Val Val Leu Leu
20 25 30

Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr
35 40 45

Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile
50 55 60

Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp
65 70 75 80

Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile
85 90 95

Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala
100 105 110

Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu
115 120 125

Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr
130 135 140

Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His
145 150 155 160

Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys
165 170 175

Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu
180 185 190

Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu
195 200 205

Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala
210 215 220

Thr Val Ala Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly
225 230 235 240

Glu Glu Pro Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro
245 250 255

Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala
260 265 270

Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser
275 280 285

Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala
290 295 300

Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro
305 310 315 320

Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr
325 330 335

Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln
340 345 350

Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln
355 360 365

Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg

370 375 380

Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser
385 390 395 400

Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn
405 410 415

Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val
420 425 430

Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser
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Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser
450 455 460

Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn
465 470 475 480

Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp
485 490 495

Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln
500 505 510

Glu Gly Gln Gly Ala Gly Ser Lys Val His Glu Phe Gln Thr Leu Ser
515 520 525

Pro Glu Gly Ser Gly Asn Leu Ala Val Ile Gly Gly Val Ala Val Gly
530 535 540

Val Val Leu Leu Leu Val Leu Ala Gly Val Gly Phe Phe Ile His Arg
545 550 555 560

Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe
565 570 575

Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His
580 585 590

Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile
595 600 605

His Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe

610 615 620

Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
625 630 635 640

Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln
645 650 655

Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His
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His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met
675 680 685

Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu
690 695 700

Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu
705 710 715 720

Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val
725 730 735

His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val
740 745 750

Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro
755 760 765

Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr
770 775 780

Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
785 790 795 800

Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
805 810 815

Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
820 825 830

Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
835 840 845

Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
850 855 860

Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
865 870 875 880

Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro
885 890 895

Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
900 905 910

Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala
915 920 925

Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
930 935 940

Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
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Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly
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gagctccagg cagcccgcgc ctgcttcgcc ctgctgtggg gctgtgcgct ggccgcggcc 240

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aagctgaacg tggaggagcg ctccgtgggg ccgctcaccc gcaaaggctt ctacctggcc 720

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gctgtcggtg tggtcctgct tctggtgctg gcaggagttg gcttctttat ccaccgcagg 1860
aggaagaacc agcgtgcccg ccagtccccg gaggacgttt acttctccaa gtcagaacaa 1920
ctgaagcccc tgaagacata cgtggacccc cacacatatg aggaccccaa ccaggctgtg 1980
ttgaagttca ctaccgagat ccatccatcc tgtgtcactc ggcagaaggt gatcggagca 2040
ggagagtttg gggaggtgta caagggcatg ctgaagacat cctcggggaa gaaggaggtg 2100
ccggtggcca tcaagacgct gaaagccggc tacacagaga agcagcgagt ggacttcctc 2160
ggcgaggccg gcatcatggg ccagttcagc caccacaaca tcatccgcct agagggcgtc 2220
atctccaaat acaagcccat gatgatcatc actgagtaca tggagaatgg ggccctggac 2280
aagttccttc gggagaagga tggcgagttc agcgtgctgc agctggtggg catgctgcgg 2340
ggcatcgcag ctggcatgaa gtacctggcc aacatgaact atgtgcaccg tgacctggct 2400
gcccgcaaca tcctcgtcaa cagcaacctg gtctgcaagg tgtctgactt tggcctgtcc 2460
cgcgtgctgg aggacgaccc cgaggccacc tacaccacca gtggcggcaa gatccccatc 2520
cgctggaccg ccccggaggc catttcctac cggaagttca cctctgccag cgacgtgtgg 2580
agctttggca ttgtcatgtg ggaggtgatg acctatggcg agcggcccta ctgggagttg 2640
tccaaccacg aggtgatgaa agccatcaat gatggcttcc ggctccccac acccatggac 2700

```
tgcccctccg ccatctacca gctcatgatg cagtgctggc agcaggagcg tgcccgccgc   2760

cccaagttcg ctgacatcgt cagcatcctg gacaagctca ttcgtgcccc tgactccctc   2820

aagaccctgg ctgactttga cccccgcgtg tctatccggc tccccagcac gagcggctcg   2880

gagggggtgc ccttccgcac ggtgtccgag tggctggagt ccatcaagat gcagcagtat   2940

acggagcact tcatggcggc cggctacact gccatcgaga aggtggtgca gatgaccaac   3000

gacgacatca agaggattgg ggtgcggctg cccggccacc agaagcgcat cgcctacagc   3060

ctgctgggac tcaaggacca ggtgaacact gtggggatcc ccatc                   3105
```

```
<210>  20
<211>  1035
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Description of Artificial Sequence:  Predicted fusion protein

<400>  20

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1               5                   10                  15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
            20                  25                  30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
        35                  40                  45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu Leu Gln Ala
    50                  55                  60

Ala Arg Ala Cys Phe Ala Leu Leu Trp Gly Cys Ala Leu Ala Ala Ala
65                  70                  75                  80

Ala Ala Ala Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala
                85                  90                  95

Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp
            100                 105                 110

Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val
        115                 120                 125

Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp
    130                 135                 140
```

Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr
145 150 155 160

Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu
165 170 175

Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn
180 185 190

Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu
195 200 205

Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu Asn Val
210 215 220

Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala
225 230 235 240

Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr
245 250 255

Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu
260 265 270

Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr
275 280 285

Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met
290 295 300

His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu
305 310 315 320

Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser
325 330 335

Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys
340 345 350

Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys
355 360 365

Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys
370 375 380

```
Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly
385                 390                 395                 400

Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg
                405                 410                 415

Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser
            420                 425                 430

Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro
        435                 440                 445

His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu Pro His
    450                 455                 460

Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu
465                 470                 475                 480

Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr
                485                 490                 495

Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser
            500                 505                 510

Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr
        515                 520                 525

Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg
    530                 535                 540

Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr
545                 550                 555                 560

Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala
                565                 570                 575

Gly Ser Arg Val His Glu Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly
            580                 585                 590

Asn Leu Ala Val Ile Gly Gly Val Ala Val Gly Val Val Leu Leu Leu
        595                 600                 605

Val Leu Ala Gly Val Gly Phe Phe Ile His Arg Arg Arg Lys Asn Gln
    610                 615                 620
```

Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln
625 630 635 640

Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro
645 650 655

Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val
660 665 670

Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys
675 680 685

Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile
690 695 700

Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu
705 710 715 720

Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg
725 730 735

Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu
740 745 750

Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly
755 760 765

Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala
770 775 780

Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala
785 790 795 800

Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp
805 810 815

Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr
820 825 830

Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile
835 840 845

Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile
850 855 860

Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu

865 870 875 880

Ser Asn His Glu Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro
885 890 895

Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys
900 905 910

Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser
915 920 925

Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala
930 935 940

Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser
945 950 955 960

Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys
965 970 975

Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile
980 985 990

Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val
995 1000 1005

Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly
1010 1015 1020

Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
1025 1030 1035

<210> 21
<211> 1506
<212> DNA
<213> Homo sapiens

<400> 21
cagggcaagg aagtggtact gctggacttt gctgcagctg gaggggagct cggctggctc 60

acacacccgt atggcaaagg gtgggacctg atgcagaaca tcatgaatga catgccgatc 120

tacatgtact ccgtgtgcaa cgtgatgtct ggcgaccagg acaactggct ccgcaccaac 180

tgggtgtacc gaggagaggc tgagcgtatc ttcattgagc tcaagtttac tgtacgtgac 240

tgcaacagct tccctggtgg cgccagctcc tgcaaggaga ctttcaacct ctactatgcc 300

gagtcggacc tggactacgg caccaacttc cagaagcgcc tgttcaccaa gattgacacc 360

attgcgcccg atgagatcac cgtcagcagc gacttcgagg cacgccacgt gaagctgaac 420

gtggaggagc gctccgtggg gccgctcacc cgcaaaggct tctacctggc cttccaggat 480

atcggtgcct gtgtggcgct gctctccgtc cgtgtctact acaagaagtg ccccgagctg 540

ctgcagggcc tggcccactt ccctgagacc atcgccggct ctgatgcacc ttccctggcc 600

actgtggccg gcacctgtgt ggaccatgcc gtggtgccac cgggggtgga agagccccgt 660

atgcactgtg cagtggatgg cgagtggctg gtgcccattg ggcagtgcct gtgccaggca 720

ggctacgaga aggtggagga tgcctgccag gcctgctcgc ctggattttt taagtttgag 780

gcatctgaga gcccctgctt ggagtgccct gagcacacgc tgccatcccc tgagggtgcc 840

acctcctgcg agtgtgagga aggcttcttc cgggcacctc aggacccagc gtcgatgcct 900

tgcacacgac ccccctccgc cccacactac ctcacagccg tgggcatggg tgccaaggtg 960

gagctgcgct ggacgccccc tcaggacagc gggggccgcg aggacattgt ctacagcgtc 1020

acctgcgaac agtgctggcc cgagtctggg gaatgcgggc cgtgtgaggc cagtgtgcgc 1080

tactcggagc ctcctcacgg actgacccgc accagtgtga cagtgagcga cctggagccc 1140

cacatgaact acaccttcac cgtggaggcc cgcaatggcg tctcaggcct ggtaaccagc 1200

cgcagcttcc gtactgccag tgtcagcatc aaccagacag agccccccaa ggtgaggctg 1260

gagggccgca gcaccacctc gcttagcgtc tcctggagca tcccccgcc gcagcagagc 1320

cgagtgtgga agtacgaggt cacttaccgc aagaagggag actccaacag ctacaatgtg 1380

cgccgcaccg agggtttctc cgtgaccctg gacgacctgg ccccagacac cacctacctg 1440

gtccaggtgc aggcactgac gcaggagggc caggggggccg gcagcagggt gcacgaattc 1500

cagacg 1506

<210> 22
<211> 1506
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human sequence optimized for codon usage in Listeria

<400> 22
caaggtaaag aagttgtttt attagatttt gcagcagcag gtggtgaatt aggttggtta 60

acacatccat atggtaaagg ttgggattta atgcaaaata ttatgaatga tatgccaatt 120

tatatgtata gtgtttgtaa tgttatgagt ggtgatcaag ataattggtt acgtacaaat 180

tgggtttatc gtggtgaagc agaacgtatt tttattgaat taaaatttac agttcgtgat 240

tgtaatagtt ttccaggtgg tgcaagtagt tgtaaagaaa catttaattt atattatgca 300

gaaagtgatt tagattatgg tacaaatttt caaaaacgtt tatttacaaa aattgataca 360

attgcaccag atgaaattac agttagtagt gattttgaag cacgtcatgt taaattaaat 420

gttgaagaac gtagtgttgg tccattaaca cgtaaaggtt tttatttagc atttcaagat 480

attggtgcat gtgttgcatt attaagtgtt cgtgtttatt ataaaaaatg tccagaatta 540

ttacaaggtt tagcacattt tccagaaaca attgcaggta gtgatgcacc aagtttagca 600

acagttgcag gtacatgtgt tgatcatgca gttgttccac caggtggtga agaaccacgt 660

atgcattgtg cagttgatgg tgaatggtta gttccaattg gtcaatgttt atgtcaagca 720

ggttatgaaa aagttgaaga tgcatgtcaa gcatgtagtc caggtttttt taaatttgaa 780

gcaagtgaaa gtccatgttt agaatgtcca gaacatacat taccaagtcc agaaggtgca 840

acaagttgtg aatgtgaaga aggttttttt cgtgcaccac aagatccagc aagtatgcca 900

tgtacacgtc caccaagtgc accacattat ttaacagcag ttggtatggg tgcaaaagtt 960

gaattacgtt ggacaccacc acaagatagt ggtggtcgtg aagatattgt ttatagtgtt 1020

acatgtgaac aatgttggcc agaaagtggt gaatgtggtc catgtgaagc aagtgttcgt 1080

tatagtgaac caccacatgg tttaacacgt acaagtgtta cagttagtga tttagaacca 1140

catatgaatt atacatttac agttgaagca cgtaatggtg ttagtggttt agttacaagt 1200

cgtagttttc gtacagcaag tgttagtatt aatcaaacag aaccaccaaa agttcgttta 1260

gaaggtcgta gtacaacaag tttaagtgtt agttggagta ttccaccacc acaacaaagt 1320

cgtgtttgga aatatgaagt tacatatcgt aaaaaaggtg atagtaatag ttataatgtt 1380

cgtcgtacag aaggttttag tgttacatta gatgatttag caccagatac aacatattta 1440

gttcaagttc aagcattaac acaagaaggt caaggtgcag gtagtcgtgt tcatgaattt 1500

caaaca 1506

<210> 23
<211> 502
<212> PRT
<213> Homo sapeins

<400> 23

Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu
1 5 10 15

Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln
20 25 30

Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val
35 40 45

Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg
50 55 60

Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp
65 70 75 80

Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn
85 90 95

Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys
100 105 110

Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val
115 120 125

Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg
130 135 140

Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp

145 150 155 160

Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys
165 170 175

Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala
180 185 190

Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp
195 200 205

His Ala Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala
210 215 220

Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala
225 230 235 240

Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe
245 250 255

Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His
260 265 270

Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly
275 280 285

```
Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro
    290                 295                 300

Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val
305                 310                 315                 320

Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile
                325                 330                 335

Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys
            340                 345                 350

Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu
        355                 360                 365

Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr
    370                 375                 380

Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser
385                 390                 395                 400

Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro
                405                 410                 415

Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp
            420                 425                 430

Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr
        435                 440                 445

Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu
    450                 455                 460

Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu
465                 470                 475                 480

Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg
                485                 490                 495

Val His Glu Phe Gln Thr
            500


<210>  24
<211>  1689
<212>  DNA
```

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 24
atgaaaaaaa taatgctagt ttttattaca cttatattag ttagtctacc aattgcgcaa 60

caaactgaag caaaggatgc atctgcattc aataaagaaa attcaatttc atccatggca 120

ccaccagcat ctccgcctgc aagtcctaag acgccaatcg aaaagaaaca cgcggatctc 180

gagcagggca aggaagtggt actgctggac tttgctgcag ctggagggga gctcggctgg 240

ctcacacacc cgtatggcaa agggtgggac ctgatgcaga acatcatgaa tgacatgccg 300

atctacatgt actccgtgtg caacgtgatg tctggcgacc aggacaactg gctccgcacc 360

aactgggtgt accgaggaga ggctgagcgt atcttcattg agctcaagtt tactgtacgt 420

gactgcaaca gcttccctgg tggcgccagc tcctgcaagg agactttcaa cctctactat 480

gccgagtcgg acctggacta cggcaccaac ttccagaagc gcctgttcac caagattgac 540

accattgcgc ccgatgagat caccgtcagc agcgacttcg aggcacgcca cgtgaagctg 600

aacgtggagg agcgctccgt ggggccgctc acccgcaaag gcttctacct ggccttccag 660

gatatcggtg cctgtgtggc gctgctctcc gtccgtgtct actacaagaa gtgccccgag 720

ctgctgcagg gcctggccca cttccctgag accatcgccg gctctgatgc accttccctg 780

gccactgtgg ccggcacctg tgtggaccat gccgtggtgc caccgggggg tgaagagccc 840

cgtatgcact gtgcagtgga tggcgagtgg ctggtgccca ttgggcagtg cctgtgccag 900

gcaggctacg agaaggtgga ggatgcctgc caggcctgct cgcctggatt ttttaagttt 960

gaggcatctg agagcccctg cttggagtgc cctgagcaca cgctgccatc ccctgagggt 1020

gccacctcct gcgagtgtga ggaaggcttc ttccgggcac ctcaggaccc agcgtcgatg 1080

ccttgcacac gacccccctc cgccccacac tacctcacag ccgtgggcat gggtgccaag 1140

gtggagctgc gctggacgcc ccctcaggac agcgggggcc gcgaggacat tgtctacagc 1200

gtcacctgcg aacagtgctg gcccgagtct ggggaatgcg ggccgtgtga ggccagtgtg 1260

cgctactcgg agcctcctca cggactgacc cgcaccagtg tgacagtgag cgacctggag 1320

ccccacatga actacacctt caccgtggag gcccgcaatg gcgtctcagg cctggtaacc 1380

agccgcagct tccgtactgc cagtgtcagc atcaaccaga cagagccccc caaggtgagg 1440

ctggagggcc gcagcaccac ctcgcttagc gtctcctgga gcatcccccc gccgcagcag 1500

agccgagtgt ggaagtacga ggtcacttac cgcaagaagg gagactccaa cagctacaat 1560

gtgcgccgca ccgagggttt ctccgtgacc ctggacgacc tggccccaga caccacctac 1620

ctggtccagg tgcaggcact gacgcaggag ggccaggggg ccggcagcag ggtgcacgaa 1680

ttccagacg 1689

<210> 25
<211> 563
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 25

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu Gln Gly Lys
50 55 60

Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly Trp
65 70 75 80

Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln Asn Ile Met
85 90 95

Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser Gly
100 105 110

Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu Ala
115 120 125

Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp Cys Asn Ser
130 135 140

Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr Tyr
145 150 155 160

Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu Phe
165 170 175

Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val Ser Ser Asp
180 185 190

Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg Ser Val Gly
195 200 205

Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly Ala
210 215 220

Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro Glu
225 230 235 240

Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala Gly Ser Asp
245 250 255

Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp His Ala Val
260 265 270

Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp Gly
275 280 285

Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr Glu
290 295 300

Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys Phe
305 310 315 320

Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu Pro
325 330 335

Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe Arg
340 345 350

Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro Pro Ser Ala
355 360 365

Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val Glu Leu Arg
370 375 380

Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr Ser
385 390 395 400

Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro Cys
405 410 415

Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg Thr
420 425 430

Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe Thr
435 440 445

Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser Arg Ser Phe
450 455 460

Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val Arg
465 470 475 480

Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile Pro
485 490 495

Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg Lys
500 505 510

Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe Ser
515 520 525

Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln Val
530 535 540

Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg Val His Glu
545 550 555 560

Phe Gln Thr

<210> 26
<211> 1989
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 26
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

aaaaaaataa tgctagtttt tattacactt atattagtta gtctaccaat tgcgcaacaa 300

actgaagcaa aggatgcatc tgcattcaat aaagaaaatt caatttcatc catggcacca 360

ccagcatctc cgcctgcaag tcctaagacg ccaatcgaaa agaaacacgc ggatggatcc 420

gattataaag atgatgatga taaacaaggt aaagaagttg ttttattaga ttttgcagca 480

```
gcaggtggtg aattaggttg gttaacacat ccatatggta aaggttggga tttaatgcaa     540
aatattatga atgatatgcc aatttatatg tatagtgttt gtaatgttat gagtggtgat     600
caagataatt ggttacgtac aaattgggtt tatcgtggtg aagcagaacg tatttttatt     660
gaattaaaat ttacagttcg tgattgtaat agttttccag gtggtgcaag tagttgtaaa     720
gaaacattta atttatatta tgcagaaagt gatttagatt atggtacaaa ttttcaaaaa     780
cgtttattta caaaaattga tacaattgca ccagatgaaa ttacagttag tagtgatttt     840
gaagcacgtc atgttaaatt aaatgttgaa gaacgtagtg ttggtccatt aacacgtaaa     900
ggtttttatt tagcatttca agatattggt gcatgtgttg cattattaag tgttcgtgtt     960
tattataaaa aatgtccaga attattacaa ggtttagcac attttccaga aacaattgca    1020
ggtagtgatg caccaagttt agcaacagtt gcaggtacat gtgttgatca tgcagttgtt    1080
ccaccaggtg gtgaagaacc acgtatgcat tgtgcagttg atggtgaatg gttagttcca    1140
attggtcaat gtttatgtca agcaggttat gaaaaagttg aagatgcatg tcaagcatgt    1200
agtccaggtt tttttaaatt tgaagcaagt gaaagtccat gtttagaatg tccagaacat    1260
acattaccaa gtccagaagg tgcaacaagt tgtgaatgtg aagaaggttt ttttcgtgca    1320
ccacaagatc cagcaagtat gccatgtaca cgtccaccaa gtgcaccaca ttatttaaca    1380
gcagttggta tgggtgcaaa agttgaatta cgttggacac caccacaaga tagtggtggt    1440
cgtgaagata ttgtttatag tgttacatgt gaacaatgtt ggccagaaag tggtgaatgt    1500
ggtccatgtg aagcaagtgt tcgttatagt gaaccaccac atggtttaac acgtacaagt    1560
gttacagtta gtgatttaga accacatatg aattatacat ttacagttga agcacgtaat    1620
ggtgttagtg gtttagttac aagtcgtagt tttcgtacag caagtgttag tattaatcaa    1680
acagaaccac caaaagttcg tttagaaggt cgtagtacaa caagtttaag tgttagttgg    1740
agtattccac caccacaaca aagtcgtgtt tggaaatatg aagttacata tcgtaaaaaa    1800
ggtgatagta atagttataa tgttcgtcgt acagaaggtt ttagtgttac attagatgat    1860
ttagcaccag atacaacata tttagttcaa gttcaagcat taacacaaga aggtcaaggt    1920
gcaggtagtc gtgttcatga atttcaaaca gaacaaaaat taattagtga agaagattta    1980
tgagagctc                                                            1989
```

<210> 27
<211> 581
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 27

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
50 55 60

Asp Asp Asp Asp Lys Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala
65 70 75 80

Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly
85 90 95

Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr
100 105 110

Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr
115 120 125

Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys
130 135 140

Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys
145 150 155 160

Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly
165 170 175

Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro
180 185 190

Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu
195 200 205

Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr
210 215 220

Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg
225 230 235 240

Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe
245 250 255

Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala
260 265 270

Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro
275 280 285

Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln
290 295 300

Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala
305 310 315 320

Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu
325 330 335

Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys
340 345 350

Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met
355 360 365

Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly
370 375 380

Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly
385 390 395 400

Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro
405 410 415

Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu
420 425 430

Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu
435 440 445

Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser
450 455 460

Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn
465 470 475 480

Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser
485 490 495

Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp
500 505 510

Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn
515 520 525

Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro
530 535 540

Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln
545 550 555 560

Gly Ala Gly Ser Arg Val His Glu Phe Gln Thr Glu Gln Lys Leu Ile
565 570 575

Ser Glu Glu Asp Leu
580

<210> 28
<211> 1989
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Construct for fusion protein

<400> 28
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60
atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120
atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180
gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240
aaaaaaatta tgttagtttt tattacatta attttagtta gtttaccaat tgcacaacaa 300
acagaagcaa aagatgcaag tgcatttaat aaagaaaata gtattagtag tatggcacca 360
ccagcaagtc caccagcaag tccaaaaaca ccaattgaaa aaaaacatgc agatggatcc 420
gattataaag atgatgatga taaacaaggt aaagaagttg ttttattaga ttttgcagca 480
gcaggtggtg aattaggttg gttaacacat ccatatggta aaggttggga tttaatgcaa 540
aatattatga atgatatgcc aatttatatg tatagtgttt gtaatgttat gagtggtgat 600
caagataatt ggttacgtac aaattgggtt tatcgtggtg aagcagaacg tatttttatt 660
gaattaaaat ttacagttcg tgattgtaat agttttccag gtggtgcaag tagttgtaaa 720

gaaacattta atttatatta tgcagaaagt gatttagatt atggtacaaa ttttcaaaaa 780

cgtttattta caaaaattga tacaattgca ccagatgaaa ttacagttag tagtgatttt 840

gaagcacgtc atgttaaatt aaatgttgaa gaacgtagtg ttggtccatt aacacgtaaa 900

ggtttttatt tagcatttca agatattggt gcatgtgttg cattattaag tgttcgtgtt 960

tattataaaa aatgtccaga attattacaa ggtttagcac attttccaga aacaattgca 1020

ggtagtgatg caccaagttt agcaacagtt gcaggtacat gtgttgatca tgcagttgtt 1080

ccaccaggtg gtgaagaacc acgtatgcat tgtgcagttg atggtgaatg gttagttcca 1140

attggtcaat gtttatgtca agcaggttat gaaaaagttg aagatgcatg tcaagcatgt 1200

agtccaggtt tttttaaatt tgaagcaagt gaaagtccat gtttagaatg tccagaacat 1260

acattaccaa gtccagaagg tgcaacaagt tgtgaatgtg aagaaggttt ttttcgtgca 1320

ccacaagatc cagcaagtat gccatgtaca cgtccaccaa gtgcaccaca ttatttaaca 1380

gcagttggta tgggtgcaaa agttgaatta cgttggacac caccacaaga tagtggtggt 1440

cgtgaagata ttgtttatag tgttacatgt gaacaatgtt ggccagaaag tggtgaatgt 1500

ggtccatgtg aagcaagtgt tcgttatagt gaaccaccac atggtttaac acgtacaagt 1560

gttacagtta gtgatttaga accacatatg aattatacat ttacagttga agcacgtaat 1620

ggtgttagtg gtttagttac aagtcgtagt tttcgtacag caagtgttag tattaatcaa 1680

acagaaccac caaaagttcg tttagaaggt cgtagtacaa caagtttaag tgttagttgg 1740

agtattccac caccacaaca aagtcgtgtt tggaaatatg aagttacata tcgtaaaaaa 1800

ggtgatagta atagttataa tgttcgtcgt acagaaggtt ttagtgttac attagatgat 1860

ttagcaccag atacaacata tttagttcaa gttcaagcat taacacaaga aggtcaaggt 1920

gcaggtagtc gtgttcatga atttcaaaca gaacaaaaat taattagtga agaagattta 1980

tgagagctc 1989

<210> 29
<211> 581
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted Fusion protein

<400> 29

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys

20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
50 55 60

Asp Asp Asp Asp Lys Gln Gly Lys Glu Val Val Leu Leu Asp Phe Ala
65 70 75 80

Ala Ala Gly Gly Glu Leu Gly Trp Leu Thr His Pro Tyr Gly Lys Gly
85 90 95

Trp Asp Leu Met Gln Asn Ile Met Asn Asp Met Pro Ile Tyr Met Tyr
100 105 110

Ser Val Cys Asn Val Met Ser Gly Asp Gln Asp Asn Trp Leu Arg Thr
115 120 125

Asn Trp Val Tyr Arg Gly Glu Ala Glu Arg Ile Phe Ile Glu Leu Lys
130 135 140

Phe Thr Val Arg Asp Cys Asn Ser Phe Pro Gly Gly Ala Ser Ser Cys
145 150 155 160

Lys Glu Thr Phe Asn Leu Tyr Tyr Ala Glu Ser Asp Leu Asp Tyr Gly
165 170 175

Thr Asn Phe Gln Lys Arg Leu Phe Thr Lys Ile Asp Thr Ile Ala Pro
180 185 190

Asp Glu Ile Thr Val Ser Ser Asp Phe Glu Ala Arg His Val Lys Leu
195 200 205

Asn Val Glu Glu Arg Ser Val Gly Pro Leu Thr Arg Lys Gly Phe Tyr
210 215 220

Leu Ala Phe Gln Asp Ile Gly Ala Cys Val Ala Leu Leu Ser Val Arg
225 230 235 240

Val Tyr Tyr Lys Lys Cys Pro Glu Leu Leu Gln Gly Leu Ala His Phe
245 250 255

Pro Glu Thr Ile Ala Gly Ser Asp Ala Pro Ser Leu Ala Thr Val Ala
260 265 270

Gly Thr Cys Val Asp His Ala Val Val Pro Pro Gly Gly Glu Glu Pro
        275   280   285

Arg Met His Cys Ala Val Asp Gly Glu Trp Leu Val Pro Ile Gly Gln
    290   295   300

Cys Leu Cys Gln Ala Gly Tyr Glu Lys Val Glu Asp Ala Cys Gln Ala
305   310   315   320

Cys Ser Pro Gly Phe Phe Lys Phe Glu Ala Ser Glu Ser Pro Cys Leu
                325   330   335

Glu Cys Pro Glu His Thr Leu Pro Ser Pro Glu Gly Ala Thr Ser Cys
            340   345   350

Glu Cys Glu Glu Gly Phe Phe Arg Ala Pro Gln Asp Pro Ala Ser Met
        355   360   365

Pro Cys Thr Arg Pro Pro Ser Ala Pro His Tyr Leu Thr Ala Val Gly
    370   375   380

Met Gly Ala Lys Val Glu Leu Arg Trp Thr Pro Pro Gln Asp Ser Gly
385   390   395   400

Gly Arg Glu Asp Ile Val Tyr Ser Val Thr Cys Glu Gln Cys Trp Pro
                405   410   415

Glu Ser Gly Glu Cys Gly Pro Cys Glu Ala Ser Val Arg Tyr Ser Glu
            420   425   430

Pro Pro His Gly Leu Thr Arg Thr Ser Val Thr Val Ser Asp Leu Glu
        435   440   445

Pro His Met Asn Tyr Thr Phe Thr Val Glu Ala Arg Asn Gly Val Ser
    450   455   460

Gly Leu Val Thr Ser Arg Ser Phe Arg Thr Ala Ser Val Ser Ile Asn
465   470   475   480

Gln Thr Glu Pro Pro Lys Val Arg Leu Glu Gly Arg Ser Thr Thr Ser
                485   490   495

Leu Ser Val Ser Trp Ser Ile Pro Pro Pro Gln Gln Ser Arg Val Trp
            500   505   510

Lys Tyr Glu Val Thr Tyr Arg Lys Lys Gly Asp Ser Asn Ser Tyr Asn
515 520 525

Val Arg Arg Thr Glu Gly Phe Ser Val Thr Leu Asp Asp Leu Ala Pro
530 535 540

Asp Thr Thr Tyr Leu Val Gln Val Gln Ala Leu Thr Gln Glu Gly Gln
545 550 555 560

Gly Ala Gly Ser Arg Val His Glu Phe Gln Thr Glu Gln Lys Leu Ile
565 570 575

Ser Glu Glu Asp Leu
580

<210> 30
<211> 1968
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 30
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

gcatacgaca gtcgttttga tgaatgggta cagaaactga aagaggaaag ctttcaaaac 300

aatacgtttg accgccgcaa atttattcaa ggagcgggga agattgcagg actttctctt 360

ggattaacga ttgcccagtc ggttggggcc tttggatccg attataaaga tgatgatgat 420

aaacaaggta aagaagttgt tttattagat tttgcagcag caggtggtga attaggttgg 480

ttaacacatc catatggtaa aggttgggat ttaatgcaaa atattatgaa tgatatgcca 540

atttatatgt atagtgtttg taatgttatg agtggtgatc aagataattg gttacgtaca 600

aattgggttt atcgtggtga agcagaacgt atttttattg aattaaaatt tacagttcgt 660

gattgtaata gttttccagg tggtgcaagt agttgtaaag aaacatttaa tttatattat 720

gcagaaagtg atttagatta tggtacaaat tttcaaaaac gtttatttac aaaaattgat 780

acaattgcac cagatgaaat tacagttagt agtgattttg aagcacgtca tgttaaatta 840

aatgttgaag aacgtagtgt tggtccatta acacgtaaag gtttttattt agcatttcaa 900

gatattggtg catgtgttgc attattaagt gttcgtgttt attataaaaa atgtccagaa 960

ttattacaag gtttagcaca ttttccagaa acaattgcag gtagtgatgc accaagttta 1020

gcaacagttg caggtacatg tgttgatcat gcagttgttc caccaggtgg tgaagaacca 1080

cgtatgcatt gtgcagttga tggtgaatgg ttagttccaa ttggtcaatg tttatgtcaa 1140

gcaggttatg aaaaagttga agatgcatgt caagcatgta gtccaggttt ttttaaattt 1200

gaagcaagtg aaagtccatg tttagaatgt ccagaacata cattaccaag tccagaaggt 1260

gcaacaagtt gtgaatgtga agaaggtttt tttcgtgcac cacaagatcc agcaagtatg 1320

ccatgtacac gtccaccaag tgcaccacat tatttaacag cagttggtat gggtgcaaaa 1380

gttgaattac gttggacacc accacaagat agtggtggtc gtgaagatat tgtttatagt 1440

gttacatgtg aacaatgttg gccagaaagt ggtgaatgtg gtccatgtga agcaagtgtt 1500

cgttatagtg aaccaccaca tggtttaaca cgtacaagtg ttacagttag tgatttagaa 1560

ccacatatga attatacatt tacagttgaa gcacgtaatg gtgttagtgg tttagttaca 1620

agtcgtagtt ttcgtacagc aagtgttagt attaatcaaa cagaaccacc aaaagttcgt 1680

ttagaaggtc gtagtacaac aagtttaagt gttagttgga gtattccacc accacaacaa 1740

agtcgtgttt ggaaatatga agttacatat cgtaaaaaag gtgatagtaa tagttataat 1800

gttcgtcgta cagaaggttt tagtgttaca ttagatgatt tagcaccaga tacaacatat 1860

ttagttcaag ttcaagcatt aacacaagaa ggtcaaggtg caggtagtcg tgttcatgaa 1920

tttcaaacag aacaaaaatt aattagtgaa gaagatttat gagagctc 1968

<210> 31
<211> 574
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted Fusion Protein

<400> 31

Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu
1 5 10 15

Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly
20 25 30

Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser
35 40 45

Val Gly Ala Phe Gly Ser Asp Tyr Lys Asp Asp Asp Asp Lys Gln Gly
50 55 60

Lys Glu Val Val Leu Leu Asp Phe Ala Ala Ala Gly Gly Glu Leu Gly
65 70 75 80

Trp Leu Thr His Pro Tyr Gly Lys Gly Trp Asp Leu Met Gln Asn Ile
85 90 95

Met Asn Asp Met Pro Ile Tyr Met Tyr Ser Val Cys Asn Val Met Ser
100 105 110

Gly Asp Gln Asp Asn Trp Leu Arg Thr Asn Trp Val Tyr Arg Gly Glu
115 120 125

Ala Glu Arg Ile Phe Ile Glu Leu Lys Phe Thr Val Arg Asp Cys Asn
130 135 140

Ser Phe Pro Gly Gly Ala Ser Ser Cys Lys Glu Thr Phe Asn Leu Tyr
145 150 155 160

Tyr Ala Glu Ser Asp Leu Asp Tyr Gly Thr Asn Phe Gln Lys Arg Leu
165 170 175

Phe Thr Lys Ile Asp Thr Ile Ala Pro Asp Glu Ile Thr Val Ser Ser
180 185 190

Asp Phe Glu Ala Arg His Val Lys Leu Asn Val Glu Glu Arg Ser Val
195 200 205

Gly Pro Leu Thr Arg Lys Gly Phe Tyr Leu Ala Phe Gln Asp Ile Gly
210 215 220

Ala Cys Val Ala Leu Leu Ser Val Arg Val Tyr Tyr Lys Lys Cys Pro
225 230 235 240

Glu Leu Leu Gln Gly Leu Ala His Phe Pro Glu Thr Ile Ala Gly Ser
245 250 255

Asp Ala Pro Ser Leu Ala Thr Val Ala Gly Thr Cys Val Asp His Ala
260 265 270

Val Val Pro Pro Gly Gly Glu Glu Pro Arg Met His Cys Ala Val Asp
275 280 285

Gly Glu Trp Leu Val Pro Ile Gly Gln Cys Leu Cys Gln Ala Gly Tyr
290 295 300

Glu Lys Val Glu Asp Ala Cys Gln Ala Cys Ser Pro Gly Phe Phe Lys
305 310 315 320

Phe Glu Ala Ser Glu Ser Pro Cys Leu Glu Cys Pro Glu His Thr Leu
325 330 335

Pro Ser Pro Glu Gly Ala Thr Ser Cys Glu Cys Glu Glu Gly Phe Phe
340 345 350

Arg Ala Pro Gln Asp Pro Ala Ser Met Pro Cys Thr Arg Pro Pro Ser
355 360 365

Ala Pro His Tyr Leu Thr Ala Val Gly Met Gly Ala Lys Val Glu Leu
370 375 380

Arg Trp Thr Pro Pro Gln Asp Ser Gly Gly Arg Glu Asp Ile Val Tyr
385 390 395 400

Ser Val Thr Cys Glu Gln Cys Trp Pro Glu Ser Gly Glu Cys Gly Pro
405 410 415

Cys Glu Ala Ser Val Arg Tyr Ser Glu Pro Pro His Gly Leu Thr Arg
420 425 430

Thr Ser Val Thr Val Ser Asp Leu Glu Pro His Met Asn Tyr Thr Phe
435 440 445

Thr Val Glu Ala Arg Asn Gly Val Ser Gly Leu Val Thr Ser Arg Ser
450 455 460

Phe Arg Thr Ala Ser Val Ser Ile Asn Gln Thr Glu Pro Pro Lys Val
465 470 475 480

Arg Leu Glu Gly Arg Ser Thr Thr Ser Leu Ser Val Ser Trp Ser Ile
485 490 495

Pro Pro Pro Gln Gln Ser Arg Val Trp Lys Tyr Glu Val Thr Tyr Arg
500 505 510

Lys Lys Gly Asp Ser Asn Ser Tyr Asn Val Arg Arg Thr Glu Gly Phe
515 520 525

Ser Val Thr Leu Asp Asp Leu Ala Pro Asp Thr Thr Tyr Leu Val Gln
530 535 540

Val Gln Ala Leu Thr Gln Glu Gly Gln Gly Ala Gly Ser Arg Val His
545 550 555 560

Glu Phe Gln Thr Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
565 570

<210> 32
<211> 1254
<212> DNA
<213> Homo sapiens

<400> 32
caccgcagga ggaagaacca gcgtgcccgc cagtccccgg aggacgttta cttctccaag 60

tcagaacaac tgaagcccct gaagacatac gtggaccccc acacatatga ggaccccaac 120
caggctgtgt tgaagttcac taccgagatc catccatcct gtgtcactcg gcagaaggtg 180
atcggagcag gagagtttgg ggaggtgtac aagggcatgc tgaagacatc ctcggggaag 240
aaggaggtgc cggtggccat caagacgctg aaagccggct acacagagaa gcagcgagtg 300
gacttcctcg gcgaggccgg catcatgggc cagttcagcc accacaacat catccgccta 360
gagggcgtca tctccaaata caagcccatg atgatcatca ctgagtacat ggagaatggg 420
gccctggaca agttccttcg ggagaaggat ggcgagttca gcgtgctgca gctggtgggc 480
atgctgcggg gcatcgcagc tggcatgaag tacctggcca acatgaacta tgtgcaccgt 540
gacctggctg cccgcaacat cctcgtcaac agcaacctgg tctgcaaggt gtctgacttt 600
ggcctgtccc gcgtgctgga ggacgacccc gaggccacct acaccaccag tggcggcaag 660
atccccatcc gctggaccgc cccggaggcc atttcctacc ggaagttcac ctctgccagc 720
gacgtgtgga gctttggcat tgtcatgtgg gaggtgatga cctatggcga gcggccctac 780
tgggagttgt ccaaccacga ggtgatgaaa gccatcaatg atggcttccg gctccccaca 840
cccatggact gcccctccgc catctaccag ctcatgatgc agtgctggca gcaggagcgt 900
gcccgccgcc ccaagttcgc tgacatcgtc agcatcctgg acaagctcat tcgtgcccct 960
gactccctca agaccctggc tgactttgac cccgcgtgt ctatccggct ccccagcacg 1020
agcggctcgg agggggtgcc cttccgcacg gtgtccgagt ggctggagtc catcaagatg 1080
cagcagtata cggagcactt catggcggcc ggctacactg ccatcgagaa ggtggtgcag 1140
atgaccaacg acgacatcaa gaggattggg gtgcggctgc ccggccacca gaagcgcatc 1200
gcctacagcc tgctgggact caaggaccag gtgaacactg tggggatccc catc 1254

<210> 33
<211> 1254

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Sequence Optimized for codon usage in Listeria

<400> 33
cacagacgta gaaaaaatca acgtgctcga caatccccag aagatgtgta tttttcgaaa 60

agtgaacaat taaaaccatt aaaaacttat gttgatccgc atacgtacga agacccaaat 120

caagcagtat taaaatttac aacagaaata cacccaagtt gtgttacaag acaaaaagtt 180

attggagcag gtgaattcgg agaggtatat aaaggtatgt taaaaacatc atcaggtaaa 240

aaagaagttc cggttgcaat taaaacctta aaggcaggat atacagaaaa acagcgagtt 300

gatttttttag gtgaagcagg aattatgggt caatttagcc atcataatat tattcgtttg 360

gaaggagtaa taagtaaata taaaccaatg atgattatta cagaatacat ggaaaacggt 420

gctttagata aatttttacg tgaaaaggat ggtgaattta gtgttttaca attggttggt 480

atgttaagag gaattgctgc aggtatgaaa tatttagcta atatgaatta tgttcaccgt 540

gatttggcag caagaaatat cctagtcaat tccaatttag tatgtaaagt tagtgatttt 600

ggtttaagca gagtattaga agacgatcca gaggcaacct atacaacatc gggaggtaaa 660

attcctattc gttggacagc accagaagct atcagttacc gtaaatttac aagtgcatca 720

gacgtgtgga gttttgggat tgtaatgtgg gaagttatga catatggaga aagaccatat 780

tgggaattaa gtaatcatga agttatgaaa gcaattaacg atggatttag attaccaact 840

ccgatggatt gtccatctgc catttatcaa ctaatgatgc aatgttggca acaagaaaga 900

gcacgacgtc caaaatttgc agatattgtt agtattttag acaaattaat tcgtgcacca 960

gatagtttaa aaactttagc agactttgat cctcgtgtta gtattcgatt accaagtacg 1020

tcaggttccg aaggagttcc atttcgcaca gtctccgaat ggttggaatc aattaaaatg 1080

caacaataca ccgaacactt tatggcagca ggttacacag caatcgaaaa agttgttcaa 1140

atgacaaatg atgatattaa acgtattgga gttagattac caggccacca gaaacgtatt 1200

gcatattctt tattaggttt aaaagatcaa gttaataccg tgggaattcc aatt 1254

<210> 34
<211> 456
<212> PRT
<213> Homo sapiens

<400> 34

Val His Glu Phe Gln Thr Leu Ser Pro Glu Gly Ser Gly Asn Leu Ala
1 5 10 15

Val Ile Gly Gly Val Ala Val Gly Val Val Leu Leu Leu Val Leu Ala
20 25 30

Gly Val Gly Phe Phe Ile His Arg Arg Arg Lys Asn Gln Arg Ala Arg
35 40 45

Gln Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro
50 55 60

Leu Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala
65 70 75 80

Val Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln
85 90 95

Lys Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu
100 105 110

Lys Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu
115 120 125

Lys Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala
130 135 140

Gly Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly
145 150 155 160

Val Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu
165 170 175

Asn Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser
180 185 190

Val Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys
195 200 205

Tyr Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn
210 215 220

Ile Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu
225 230 235 240

Ser Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly
245 250 255

Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg
260 265 270

Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp
275 280 285

Glu Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His
290 295 300

Glu Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met
305 310 315 320

Asp Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln
325 330 335

Glu Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp
340 345 350

Lys Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp
355 360 365

Pro Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val
370 375 380

Pro Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln
385 390 395 400

Tyr Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val
405 410 415

Val Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro
420 425 430

Gly His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln
435 440 445

Val Asn Thr Val Gly Ile Pro Ile
450 455

<210> 35
<211> 1437
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion Protein

<400> 35
atgaaaaaa taatgctagt ttttattaca cttatattag ttagtctacc aattgcgcaa 60

caaactgaag caaaggatgc atctgcattc aataaagaaa attcaatttc atccatggca 120

ccaccagcat ctccgcctgc aagtcctaag acgccaatcg aaaagaaaca cgcggatctc 180

gagcaccgca ggaggaagaa ccagcgtgcc cgccagtccc cggaggacgt ttacttctcc 240

aagtcagaac aactgaagcc cctgaagaca tacgtggacc cccacacata tgaggacccc 300

aaccaggctg tgttgaagtt cactaccgag atccatccat cctgtgtcac tcggcagaag 360

gtgatcggag caggagagtt tggggaggtg tacaaggca tgctgaagac atcctcgggg 420

aagaaggagg tgccggtggc catcaagacg ctgaaagccg gctacacaga gaagcagcga 480

gtggacttcc tcggcgaggc cggcatcatg ggccagttca gccaccacaa catcatccgc 540

ctagagggcg tcatctccaa atacaagccc atgatgatca tcactgagta catggagaat 600

ggggccctgg acaagttcct tcgggagaag gatggcgagt tcagcgtgct gcagctggtg 660

ggcatgctgc ggggcatcgc agctggcatg aagtacctgg ccaacatgaa ctatgtgcac 720

cgtgacctgg ctgcccgcaa catcctcgtc aacagcaacc tggtctgcaa ggtgtctgac 780

tttggcctgt cccgcgtgct ggaggacgac cccgaggcca cctacaccac cagtggcggc 840

aagatcccca tccgctggac cgccccggag gccatttcct accggaagtt cacctctgcc 900

agcgacgtgt ggagctttgg cattgtcatg tgggaggtga tgacctatgg cgagcggccc 960

tactgggagt tgtccaacca cgaggtgatg aaagccatca atgatggctt ccggctcccc 1020

acacccatgg actgcccctc cgccatctac cagctcatga tgcagtgctg gcagcaggag 1080

cgtgcccgcc gccccaagtt cgctgacatc gtcagcatcc tggacaagct cattcgtgcc 1140

cctgactccc tcaagaccct ggctgacttt gacccccgcg tgtctatccg gctccccagc 1200

acgagcggct cggagggggt gcccttccgc acggtgtccg agtggctgga gtccatcaag 1260

atgcagcagt atacggagca cttcatggcg gccggctaca ctgccatcga gaaggtggtg 1320

cagatgacca acgacgacat caagaggatt ggggtgcggc tgcccggcca ccagaagcgc 1380

atcgcctaca gcctgctggg actcaaggac caggtgaaca ctgtggggat ccccatc 1437

<210> 36
<211> 479
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted Protein Sequence

<400> 36

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu

1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Leu Glu His Arg Arg
50 55 60

Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe Ser
65 70 75 80

Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His Thr
85 90 95

Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile His
100 105 110

Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe Gly
115 120 125

Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu Val
130 135 140

Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln Arg
145 150 155 160

Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His His
165 170 175

Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met Met
180 185 190

Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu Arg
195 200 205

Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu Arg
210 215 220

Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val His
225 230 235 240

Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val Cys
245 250 255

```
Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro Glu
            260                 265                 270

Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala
        275                 280                 285

Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val Trp
    290                 295                 300

Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg Pro
305                 310                 315                 320

Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp Gly
                325                 330                 335

Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln Leu
            340                 345                 350

Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe Ala
        355                 360                 365

Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser Leu
    370                 375                 380

Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro Ser
385                 390                 395                 400

Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp Leu
                405                 410                 415

Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala Gly
            420                 425                 430

Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile Lys
        435                 440                 445

Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr Ser
    450                 455                 460

Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
465                 470                 475
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<210> 37
<211> 1737
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein sequence

<400> 37
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

aaaaaaataa tgctagtttt tattacactt atattagtta gtctaccaat tgcgcaacaa 300

actgaagcaa aggatgcatc tgcattcaat aaagaaaatt caatttcatc catggcacca 360

ccagcatctc cgcctgcaag tcctaagacg ccaatcgaaa agaaacacgc ggatggatcc 420

gattataaag atgatgatga taaacacaga cgtagaaaaa atcaacgtgc tcgacaatcc 480

ccagaagatg tgtatttttc gaaaagtgaa caattaaaac cattaaaaac ttatgttgat 540

ccgcatacgt acgaagaccc aaatcaagca gtattaaaat ttacaacaga aatacaccca 600

agttgtgtta caagacaaaa agttattgga gcaggtgaat tcggagaggt atataaaggt 660

atgttaaaaa catcatcagg taaaaaagaa gttccggttg caattaaaac cttaaaggca 720

ggatatacag aaaaacagcg agttgatttt ttaggtgaag caggaattat gggtcaattt 780

agccatcata atattattcg tttggaagga gtaataagta aatataaacc aatgatgatt 840

attacagaat acatggaaaa cggtgcttta gataaatttt tacgtgaaaa ggatggtgaa 900

tttagtgttt tacaattggt tggtatgtta agaggaattg ctgcaggtat gaaatattta 960

gctaatatga attatgttca ccgtgatttg gcagcaagaa atatcctagt caattccaat 1020

ttagtatgta aagttagtga ttttggttta agcagagtat tagaagacga tccagaggca 1080

acctatacaa catcgggagg taaaattcct attcgttgga cagcaccaga agctatcagt 1140

taccgtaaat ttacaagtgc atcagacgtg tggagttttg ggattgtaat gtgggaagtt 1200

atgacatatg gagaaagacc atattgggaa ttaagtaatc atgaagttat gaaagcaatt 1260

aacgatggat ttagattacc aactccgatg gattgtccat ctgccattta tcaactaatg 1320

atgcaatgtt ggcaacaaga aagagcacga cgtccaaaat ttgcagatat tgttagtatt 1380

ttagacaaat taattcgtgc accagatagt ttaaaaactt tagcagactt tgatcctcgt 1440

gttagtattc gattaccaag tacgtcaggt tccgaaggag ttccatttcg cacagtctcc 1500

gaatggttgg aatcaattaa aatgcaacaa tacaccgaac actttatggc agcaggttac 1560

acagcaatcg aaaaagttgt tcaaatgaca aatgatgata ttaaacgtat tggagttaga 1620

ttaccaggcc accagaaacg tattgcatat tctttattag gtttaaaaga tcaagttaat 1680

accgtgggaa ttccaattga acaaaaatta atttccgaag aagacttata agagctc 1737

<210> 38
<211> 497
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 38

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
50 55 60

Asp Asp Asp Asp Lys His Arg Arg Arg Lys Asn Gln Arg Ala Arg Gln
65 70 75 80

Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro Leu
85 90 95

Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val
100 105 110

Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln Lys
115 120 125

Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu Lys
130 135 140

Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu Lys
145 150 155 160

Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala Gly
165 170 175

Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly Val
180 185 190

Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu Asn
195 200 205

Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser Val
210 215 220

Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys Tyr
225 230 235 240

Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
245 250 255

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser
260 265 270

Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly Gly
275 280 285

Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg Lys
290 295 300

Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu
305 310 315 320

Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu
325 330 335

Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp
340 345 350

Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu
355 360 365

Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys
370 375 380

Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro
385 390 395 400

Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro
405 410 415

Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr
420 425 430

Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val
435 440 445

Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly
450 455 460

His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val
465 470 475 480

Asn Thr Val Gly Ile Pro Ile Glu Gln Lys Leu Ile Ser Glu Glu Asp
485 490 495

Leu

<210> 39
<211> 1737
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 39
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

aaaaaaatta tgttagtttt tattacatta attttagtta gtttaccaat tgcacaacaa 300

acagaagcaa aagatgcaag tgcatttaat aaagaaaata gtattagtag tatggcacca 360

ccagcaagtc caccagcaag tccaaaaaca ccaattgaaa aaaaacatgc agatggatcc 420

gattataaag acgatgatga taaacacaga cgtagaaaaa atcaacgtgc tcgacaatcc 480

ccagaagatg tgtatttttc gaaaagtgaa caattaaaac cattaaaaac ttatgttgat 540

ccgcatacgt acgaagaccc aaatcaagca gtattaaaat ttacaacaga aatacaccca 600

agttgtgtta caagacaaaa agttattgga gcaggtgaat tcggagaggt atataaaggt 660

atgttaaaaa catcatcagg taaaaaagaa gttccggttg caattaaaac cttaaaggca 720

ggatatacag aaaaacagcg agttgatttt ttaggtgaag caggaattat gggtcaattt 780

agccatcata atattattcg tttggaagga gtaataagta aatataaacc aatgatgatt 840

attacagaat acatggaaaa cggtgcttta gataaatttt tacgtgaaaa ggatggtgaa 900

tttagtgttt tacaattggt tggtatgtta agaggaattg ctgcaggtat gaaatattta 960

gctaatatga attatgttca ccgtgatttg gcagcaagaa atatcctagt caattccaat 1020

ttagtatgta aagttagtga ttttggttta agcagagtat tagaagacga tccagaggca 1080

acctatacaa catcgggagg taaaattcct attcgttgga cagcaccaga agctatcagt 1140

taccgtaaat ttacaagtgc atcagacgtg tggagttttg ggattgtaat gtgggaagtt 1200

atgacatatg gagaaagacc atattgggaa ttaagtaatc atgaagttat gaaagcaatt 1260

aacgatggat ttagattacc aactccgatg gattgtccat ctgccattta tcaactaatg 1320

atgcaatgtt ggcaacaaga aagagcacga cgtccaaaat ttgcagatat tgttagtatt 1380

ttagacaaat taattcgtgc accagatagt ttaaaaactt tagcagactt tgatcctcgt 1440

gttagtattc gattaccaag tacgtcaggt tccgaaggag ttccatttcg cacagtctcc 1500

gaatggttgg aatcaattaa aatgcaacaa tacaccgaac actttatggc agcaggttac 1560

acagcaatcg aaaaagttgt tcaaatgaca aatgatgata ttaaacgtat tggagttaga 1620

ttaccaggcc accagaaacg tattgcatat tctttattag gtttaaaaga tcaagttaat 1680

accgtgggaa ttccaattga acaaaaatta atttccgaag aagacttata agagctc 1737

<210> 40
<211> 497
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted Fusion Protein

<400> 40

Met Lys Lys Ile Met Leu Val Phe Ile Thr Leu Ile Leu Val Ser Leu
1 5 10 15

Pro Ile Ala Gln Gln Thr Glu Ala Lys Asp Ala Ser Ala Phe Asn Lys
20 25 30

Glu Asn Ser Ile Ser Ser Met Ala Pro Pro Ala Ser Pro Pro Ala Ser
35 40 45

Pro Lys Thr Pro Ile Glu Lys Lys His Ala Asp Gly Ser Asp Tyr Lys
50 55 60

Asp Asp Asp Asp Lys His Arg Arg Arg Lys Asn Gln Arg Ala Arg Gln
65 70 75 80

Ser Pro Glu Asp Val Tyr Phe Ser Lys Ser Glu Gln Leu Lys Pro Leu
85 90 95

Lys Thr Tyr Val Asp Pro His Thr Tyr Glu Asp Pro Asn Gln Ala Val
100 105 110

Leu Lys Phe Thr Thr Glu Ile His Pro Ser Cys Val Thr Arg Gln Lys
115 120 125

Val Ile Gly Ala Gly Glu Phe Gly Glu Val Tyr Lys Gly Met Leu Lys
130 135 140

Thr Ser Ser Gly Lys Lys Glu Val Pro Val Ala Ile Lys Thr Leu Lys
145 150 155 160

Ala Gly Tyr Thr Glu Lys Gln Arg Val Asp Phe Leu Gly Glu Ala Gly
165 170 175

Ile Met Gly Gln Phe Ser His His Asn Ile Ile Arg Leu Glu Gly Val
180 185 190

Ile Ser Lys Tyr Lys Pro Met Met Ile Ile Thr Glu Tyr Met Glu Asn
195 200 205

Gly Ala Leu Asp Lys Phe Leu Arg Glu Lys Asp Gly Glu Phe Ser Val
210 215 220

Leu Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys Tyr
225 230 235 240

Leu Ala Asn Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn Ile
245 250 255

Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu Ser
260 265 270

Arg Val Leu Glu Asp Asp Pro Glu Ala Thr Tyr Thr Thr Ser Gly Gly
275 280 285

Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ser Tyr Arg Lys
290 295 300

Phe Thr Ser Ala Ser Asp Val Trp Ser Phe Gly Ile Val Met Trp Glu
305 310 315 320

Val Met Thr Tyr Gly Glu Arg Pro Tyr Trp Glu Leu Ser Asn His Glu
325 330 335

Val Met Lys Ala Ile Asn Asp Gly Phe Arg Leu Pro Thr Pro Met Asp

340 345 350

Cys Pro Ser Ala Ile Tyr Gln Leu Met Met Gln Cys Trp Gln Gln Glu
355 360 365

Arg Ala Arg Arg Pro Lys Phe Ala Asp Ile Val Ser Ile Leu Asp Lys
370 375 380

Leu Ile Arg Ala Pro Asp Ser Leu Lys Thr Leu Ala Asp Phe Asp Pro
385 390 395 400

Arg Val Ser Ile Arg Leu Pro Ser Thr Ser Gly Ser Glu Gly Val Pro
405 410 415

Phe Arg Thr Val Ser Glu Trp Leu Glu Ser Ile Lys Met Gln Gln Tyr
420 425 430

Thr Glu His Phe Met Ala Ala Gly Tyr Thr Ala Ile Glu Lys Val Val
435 440 445

Gln Met Thr Asn Asp Asp Ile Lys Arg Ile Gly Val Arg Leu Pro Gly
450 455 460

His Gln Lys Arg Ile Ala Tyr Ser Leu Leu Gly Leu Lys Asp Gln Val
465 470 475 480

Asn Thr Val Gly Ile Pro Ile Glu Gln Lys Leu Ile Ser Glu Glu Asp
485 490 495

Leu

<210> 41
<211> 1716
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion protein construct

<400> 41
ggtacctcct ttgattagta tattcctatc ttaaagttac ttttatgtgg aggcattaac 60

atttgttaat gacgtcaaaa ggatagcaag actagaataa agctataaag caagcatata 120

atattgcgtt tcatctttag aagcgaattt cgccaatatt ataattatca aaagagaggg 180

gtggcaaacg gtatttggca ttattaggtt aaaaaatgta gaaggagagt gaaacccatg 240

gcatacgaca gtcgttttga tgaatgggta cagaaactga aagaggaaag ctttcaaaac 300

aatacgtttg accgccgcaa atttattcaa ggagcgggga agattgcagg actttctctt 360

ggattaacga ttgcccagtc ggttggggcc tttggatccg attataaaga tgatgatgat 420

aaacacagac gtagaaaaaa tcaacgtgct cgacaatccc cagaagatgt gtatttttcg 480

aaaagtgaac aattaaaacc attaaaaact tatgttgatc cgcatacgta cgaagaccca 540

aatcaagcag tattaaaatt tacaacagaa atacacccaa gttgtgttac aagacaaaaa 600

gttattggag caggtgaatt cggagaggta tataaaggta tgttaaaaac atcatcaggt 660

aaaaaagaag ttccggttgc aattaaaacc ttaaaggcag gatatacaga aaaacagcga 720

gttgattttt taggtgaagc aggaattatg ggtcaattta gccatcataa tattattcgt 780

ttggaaggag taataagtaa atataaacca atgatgatta ttacagaata catggaaaac 840

ggtgctttag ataaattttt acgtgaaaag gatggtgaat ttagtgtttt acaattggtt 900

ggtatgttaa gaggaattgc tgcaggtatg aaatatttag ctaatatgaa ttatgttcac 960

cgtgatttgg cagcaagaaa tatcctagtc aattccaatt tagtatgtaa agttagtgat 1020

tttggtttaa gcagagtatt agaagacgat ccagaggcaa cctatacaac atcgggaggt 1080

aaaattccta ttcgttggac agcaccagaa gctatcagtt accgtaaatt tacaagtgca 1140

tcagacgtgt ggagttttgg gattgtaatg tgggaagtta tgacatatgg agaaagacca 1200

tattgggaat taagtaatca tgaagttatg aaagcaatta acgatggatt tagattacca 1260

actccgatgg attgtccatc tgccatttat caactaatga tgcaatgttg gcaacaagaa 1320

agagcacgac gtccaaaatt tgcagatatt gttagtattt tagacaaatt aattcgtgca 1380

ccagatagtt taaaaacttt agcagacttt gatcctcgtg ttagtattcg attaccaagt 1440

acgtcaggtt ccgaaggagt tccatttcgc acagtctccg aatggttgga atcaattaaa 1500

atgcaacaat acaccgaaca ctttatggca gcaggttaca cagcaatcga aaaagttgtt 1560

caaatgacaa atgatgatat taaacgtatt ggagttagat taccaggcca ccagaaacgt 1620

attgcatatt ctttattagg tttaaaagat caagttaata ccgtgggaat tccaattgaa 1680

caaaaattaa tttccgaaga agacttataa gagctc 1716

<210> 42
<211> 490
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Predicted fusion protein

<400> 42

Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu

1 5 10 15

Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly
20 25 30

Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser

35 40 45

Val Gly Ala Phe Gly Ser Asp Tyr Lys Asp Asp Asp Asp Lys His Arg
50 55 60

Arg Arg Lys Asn Gln Arg Ala Arg Gln Ser Pro Glu Asp Val Tyr Phe
65 70 75 80

Ser Lys Ser Glu Gln Leu Lys Pro Leu Lys Thr Tyr Val Asp Pro His
85 90 95

Thr Tyr Glu Asp Pro Asn Gln Ala Val Leu Lys Phe Thr Thr Glu Ile
100 105 110

His Pro Ser Cys Val Thr Arg Gln Lys Val Ile Gly Ala Gly Glu Phe
115 120 125

Gly Glu Val Tyr Lys Gly Met Leu Lys Thr Ser Ser Gly Lys Lys Glu
130 135 140

Val Pro Val Ala Ile Lys Thr Leu Lys Ala Gly Tyr Thr Glu Lys Gln
145 150 155 160

Arg Val Asp Phe Leu Gly Glu Ala Gly Ile Met Gly Gln Phe Ser His
165 170 175

His Asn Ile Ile Arg Leu Glu Gly Val Ile Ser Lys Tyr Lys Pro Met
180 185 190

Met Ile Ile Thr Glu Tyr Met Glu Asn Gly Ala Leu Asp Lys Phe Leu
195 200 205

Arg Glu Lys Asp Gly Glu Phe Ser Val Leu Gln Leu Val Gly Met Leu
210 215 220

Arg Gly Ile Ala Ala Gly Met Lys Tyr Leu Ala Asn Met Asn Tyr Val
225 230 235 240

His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Asn Ser Asn Leu Val

245 250 255

Cys Lys Val Ser Asp Phe Gly Leu Ser Arg Val Leu Glu Asp Asp Pro
260 265 270

Glu Ala Thr Tyr Thr Thr Ser Gly Gly Lys Ile Pro Ile Arg Trp Thr
275 280 285

Ala Pro Glu Ala Ile Ser Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val
290 295 300

Trp Ser Phe Gly Ile Val Met Trp Glu Val Met Thr Tyr Gly Glu Arg
305 310 315 320

Pro Tyr Trp Glu Leu Ser Asn His Glu Val Met Lys Ala Ile Asn Asp
325 330 335

Gly Phe Arg Leu Pro Thr Pro Met Asp Cys Pro Ser Ala Ile Tyr Gln
340 345 350

Leu Met Met Gln Cys Trp Gln Gln Glu Arg Ala Arg Arg Pro Lys Phe
355 360 365

Ala Asp Ile Val Ser Ile Leu Asp Lys Leu Ile Arg Ala Pro Asp Ser
370 375 380

Leu Lys Thr Leu Ala Asp Phe Asp Pro Arg Val Ser Ile Arg Leu Pro
385 390 395 400

Ser Thr Ser Gly Ser Glu Gly Val Pro Phe Arg Thr Val Ser Glu Trp
405 410 415

Leu Glu Ser Ile Lys Met Gln Gln Tyr Thr Glu His Phe Met Ala Ala
420 425 430

Gly Tyr Thr Ala Ile Glu Lys Val Val Gln Met Thr Asn Asp Asp Ile
435 440 445

Lys Arg Ile Gly Val Arg Leu Pro Gly His Gln Lys Arg Ile Ala Tyr
450 455 460

Ser Leu Leu Gly Leu Lys Asp Gln Val Asn Thr Val Gly Ile Pro Ile
465 470 475 480

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
485 490

<210> 43
<211> 9808
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion Protein Construct

<400> 43
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<213> Listeria monocytogenes

<400> 44

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<210> 45
<211> 59
<212> PRT
<213> Listeria monocytogenes

<400> 45

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1 5 10 15

Glu Asn Lys Gly Met Thr Arg Arg Glu Met Leu Lys Leu Ser Ala Val
20 25 30

Ala Gly Thr Gly Ile Ala Val Gly Ala Thr Gly Leu Gly Thr Ile Leu
35 40 45

Asn Val Val Asp Gln Val Asp Lys Ala Leu Thr
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<210> 46
<211> 53
<212> PRT
<213> Bacillus subtillus

<400> 46

Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu
1 5 10 15

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Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser
35 40 45

Val Gly Ala Phe Gly
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<220>
<223> Description of Artificial Sequence: Primer

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<210> 48
<211> 24
<212> DNA
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<220>
<223> Description of Artificial Sequence: Primer

<400> 48
acataatcag tccaaagtag atgc 24

<210> 49
<211> 29

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 49
ctctggtacc tcctttgatt agtatattc 29

<210> 50
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 50
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<210> 51
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Epitope insert

<400> 51
gattataaag atgatgatga taaa 24

<210> 52
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Epitope

<400> 52

Asp Tyr Lys Asp Asp Asp Asp Lys
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<210> 53

<211> 30
<212> DNA
<213> Artificial Sequence

<220>
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<400> 53
gaacaaaaat taattagtga agaagattta 30

<210> 54
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Epitope

<400> 54

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
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<210> 55
<211> 9
<212> PRT
<213> Mus sp.

<400> 55

Ser Pro Ser Tyr Val Tyr His Gln Phe
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<210> 56
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Epitope

<400> 56

Ser Pro Ser Tyr Ala Tyr His Gln Phe
1 5

<210> 57
<211> 29
<212> DNA
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<220>
<223> Description of Artificial Sequence: Primer

<400> 57
ctctggtacc tcctttgatt agtatattc 29

<210> 58
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 58
caatggatcc ctcgagatca taatttactt catccc 36

<210> 59
<211> 32
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<210> 60
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<210> 61
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<220>
<223> Description of Artificial Sequence: Epitope insert

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<212> DNA
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<210> 65
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

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<210> 66
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<210> 67
<211> 19
<212> DNA
<213> Listeria monocytogenes

<400> 67
aaggagagtg aaacccatg 19

<210> 68
<211> 240
<212> DNA
<213> Listeria monocytogenes

<400> 68
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<210> 69
<211> 240
<212> DNA
<213> Listeria monocytogenes

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<210> 70
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<212> PRT
<213> Listeria monocytogenes

<400> 70

Thr Glu Ala Lys Asp
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<210> 71
<211> 5
<212> PRT
<213> Listeria monocytogenes

<400> 71

Asp Lys Ala Leu Thr
1 5

<210> 72
<211> 5
<212> PRT
<213> Bacillus subtillus

<400> 72

Val Gly Ala Phe Gly
1 5

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1

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(19) World Intellectual Property Organization
International Bureau

PCT

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: LISTERIA-BASED EPHA2 VACCINES

(57) Abstract: The present invention relates to methods and compositions designed for the treatment, management, or prevention of cancer, particularly metastatic cancer and cancers of T cell origin, and hyperproliferative diseases involving EphA2-expressing cells. The methods of the invention entail the use of a *Listeria*-based EphA2 vaccine. The invention also provides pharmaceutical compositions comprising one or more *Listeria*-based vaccines of the invention either alone in combination with one or more other agents useful for cancer therapy. In certain aspects of the invention, the method entail eliciting both $CD4^+$ and $CD8^+$ T-cell responses against EphA2 and/or EphA2-expressing cells.

WO 2005/037233 A3

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/102; C12N 1/20; C07K 1/100
US CL : 424/200.1, 234.1; 435/172.1, 252.3; 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/200.1, 234.1; 435/172.1, 252.3; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01/12840 A2 (PURDUE RESEARCH FOUNDATION) 22 February 2001 (22.02.2001), see entire document.	1-39

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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